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EFFECT OF COLD-STORAGE TEMPERATURES UPON THE MEDITERRANEAN FRUIT FLY

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INTRODUCTION

Since the introduction of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) into the Hawaiian Islands and the subsequent quarantines against Hawaiian fruits, the problem of the fruit grower in these islands has been how to use his fruit to advantage at home. Many host fruits of the fruit fly are ruined long before they are suitable for either the table or storage. There are, however, other fruits, such as the avocado (*Persea gratissima*) and certain varieties of mangos (*Mangifera indica*) and star-apples (*Chrysophyllum [illegible]into*), which, while often becoming too badly infested to be of use if left to ripen normally upon the tree, become infested so late in their development that they may be preserved for commerce if they respond favorably to cold storage, and if such cold storage kills whatever stages of the fruit fly may be present in the fruit when picked.

The experimental work reported in this paper was undertaken primarily with the hope that it would be an aid in solving the discouraging problems of the local horticulturists. But whatever its value in this direction, it now appears that the results may be of much greater commercial importance in defining the conditions under which cold-storage temperatures will kill the fruit fly in stored fruits, thus rendering them free from danger as transporters of this pest from one country to another or even from one infested district to another in host fruits.

HISTORICAL REVIEW

Cold-storage temperatures have been used in economic entomology in the past more to suspend insect activity than to cause death, except in the case of the Mediterranean fruit-fly work in Australia and Africa. The first practical use of cold-storage temperatures known to the writers was made by the manager of a large storage-warehouse company of Washington, D. C., in an attempt to find a safe method of protecting clothing from insect ravages during the warmer period of the year. At

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the suggestion and with the assistance of Dr. L. O. Howard experiments were carried on to determine the effect of cold-storage temperatures upon still other insects affecting stored goods. Dr. Howard (1),[1] in a paper read before the eighth annual meeting of the Association of Economic Entomologists in 1896, discussed for the first time in professional entomological literature the important use to which cold-storage temperatures may be put in controlling insects. In 1905 Duvel (2), while investigating the storage of cowpeas (*Vigna sinensis*), found that storage at 32° to 34° F. was entirely practicable and economical in combating the common bean weevil (*Bruchus obtectus*), the cowpea weevil (*Bruchus chinensis*), and the four-spotted bean weevil (*Bruchus quadrimaculatus*).

While the work referred to above was carried on primarily to safeguard produce and stored goods from attack during certain periods when pests are active, experiments to determine the effect of cold-storage temperatures upon the Mediterranean fruit fly have been undertaken with the object of killing the various stages within the fruit. The interest in this work in Africa and Australia has grown out of the fact that the growers have sought for their surplus fruit markets in northern Europe, England, and North America, and even in South America, China, and the Hawaiian Islands. To reach these markets their fruits must be in transit a sufficiently long time for infestations overlooked at the packing houses to cause considerable decay unless the cold-storage temperature to which the fruit is subjected en route either suspends or kills chance cases of infestation.

In 1906, Fuller (3) recorded the resistance of fruit-fly larvæ in a certain lot of peaches in Natal to 40° F. for 124 days. The writers question the accuracy of this statement, as they have been unable at this temperature to keep larvæ or eggs alive for more than 22 days, in tests covering several thousand larvæ and eggs (see Table I). Fuller believes from his observation that cold storage as a method of substitution for quarantines involves considerable risk.

Lounsbury (4) states in 1907 that experiments conducted by him in South Africa indicate that a temperature of 38° to 40°, continued for three weeks, is sufficient to insure the death of all fruit-fly larvæ in infested fruit, that two weeks at such a temperature causes considerable mortality, and that one week is thoroughly ineffective. In 1908, in a second paper (6), he records no living larvæ among 511 specimens found in peaches held for 21 and 27 days at 38° to 40°. It is his belief that the storage temperature necessary for the preservation of fruit in transit from Africa to countries of the Northern Hemisphere and to America is amply low to effect the extinction of all life in larvæ and eggs of the fruit fly contained within it.

Hooper (5) recorded in 1907 in West Australia that he had found that larvæ and eggs of the fruit fly could not resist temperatures ranging from

[1] Reference is made by number to "Literature cited, p. 665–666.

33° to 35° for more than 15 days, and advised that fruit kept within this range of temperature for three weeks would be perfectly free from living forms. His report indicates that the work was done carefully.

The work of Wilcox and Hunn (7) in 1914 has shown that such semi-tropical host fruits as the star-apple, fig (*Ficus* spp.), papaya (*Carica papaya*), mango, and avocado withstand without injury to texture or flavor a temperature slightly above 32° for from 27 days in the case of papaya to two months in the case of the avocado. Such periods at 32° are well above the margin of safety for complete mortality of the larvæ and eggs of the fruit fly.

EXPERIMENTAL WORK

In determining the effect of cold-storage temperatures upon the eggs and larvæ of the Mediterranean fruit fly, the writers have been fortunate in securing the cooperation of an ice company during 1913 and of an electric company during 1914 and 1915. At the cold-storage plants of these companies there were to be had all the facilities found in modern, well-regulated cold-storage plants. While an abundance of fruit-fly material is to be had in and about Honolulu, the writers have preferred in their work to infest in the insectary host fruits known to be previously free from attack. As no such fruits can be found in Hawaii under natural conditions, apples (*Malus* spp.) from California were used. These fruits were suspended for several hours in jars containing several hundred ovipositing fruit flies and then removed and held in the insectary for the number of days which experience had shown was necessary for the flies within to reach the stages desired for experiment. In this way larger amounts of material in definite stages could be used at one time than otherwise. While much of the data recorded in Table I was secured from fruit flies in apples, a sufficient amount, including observations on many thousands of eggs and larvæ, has been secured from fruit flies in peaches and kamani nuts (*Terminalia catappa*), as checks, to prove that there is no probability that the nature of the host fruit affects the action of temperatures.

No examination of material to determine the effect of various temperatures was made until the host fruits had been removed from storage from 24 to 48 hours. By placing the host fruits within storage the eggs and larvæ were under normal conditions. On examination the eggs were dissected out of the punctures and placed in moist chambers where all that hatched might be recorded. Larvæ found torpid though normal in color on examination within 24 to 48 hours after removal from storage invariably failed to resume activity.

THE EGG

No eggs hatch in cold storage if held at temperatures below 50° F. A temperature of 32° proved quickly fatal to eggs. A total of 6,747 eggs were under observation. No eggs hatched upon removal from

storage after the ninth day of refrigeration. Only one egg hatched on the ninth day, and but 2 out of 2,327 removed on the seventh, eighth and ninth days. After the tenth to fifteenth days of refrigeration, 2,221 eggs were removed to warmer temperature, but none hatched. Mortality increased rapidly after the fourth day of refrigeration; thus, on the fifth day only 15 out of 735 eggs hatched. (See Table I.)

TABLE I.—*Effect of cold-storage temperatures upon eggs and larvæ of the Mediterranean fruit fly*

Number of days in cold storage.	Temperature of storage room.	Eggs.		Larvæ.					
				First instar.		Second instar.		Third instar.	
		Number under observation.	Number hatching after removal from storage.	Number alive.	Number dead.	Number alive.	Number dead.	Number alive.	Number dead.
	°F.								
1	32	81	81	.....	.....	252	40	33	7
2	32	528	520	94	0	463	9	53	2
3	32	150	135	37	1	226	15	16	75
4	32	336	216	285	26	152	0	101	3
5	32	735	15	19	202	71	175	.....	.....
6	32	469	12	26	165	18	50	105	10
7	32	659	1	11	454	14	64	135	132
8	32	834	0	2	845	20	423	38	200
9	32	734	1	0	339	11	473	20	429
10	32	.....	.....	0	701	0	257	.....	.....
11	32	635	0	0	450	0	332	6	374
12	32	887	0	0	440	0	493	0	157
13	32	.....	.....	0	355	0	276	0	173
14	32	699	0	0	273	0	248	0	152
15	32	.....	.....	.....	.....	0	262	0	144
2	32–33	.....	.....	86	0	78	0	3	0
3	32–33	.....	.....	154	1	146	2	89	0
4	32–33	.....	.....	46	0	73	0	32	0
5	32–33	.....	.....	96	0	39	0	30	0
6	32–33	152	23	279	7	8	1	24	0
7	32–33	.....	.....	31	1	16	11	9	0
8	32–33	401	5	35	163	3	27	10	16
9	32–33	.....	.....	0	169	0	167	2	14
10	32–33	357	0	2	179	0	110	0	31
12	32–33	784	0	0	880	0	86	0	35
13	32–33	900	0	0	637	0	35	0	2
14	32–33	1,001	0	0	425	0	42	0	28
15	32–33	1,121	0	0	255	.....	.....	.....	.....
16	32–33	312	0	0	519	0	43	.....	.....
17	32–33	.....	.....	0	143	0	29	0	3
3	33–34	.....	.....	60	0	94	0	55	0
4	33–34	.....	.....	108	2	107	2	68	0
5	33–34	.....	.....	42	26	79	28	.....	.....
6	33–34	.....	.....	68	32	286	169	8	5
7	33–34	.....	.....	75	20	81	100	55	1
8	33–34	300	45	46	20	35	175	51	48
9	34–34	500	0	38	207	48	456	31	189
10	33–34	541	0	4	1,446	32	296	0	48
11	33–34	.....	.....	0	72	0	314	4	126
12	33–34	358	0	1	215	0	509	0	48
13	33–34	.....	.....	2	632	0	385	0	4

TABLE I.—*Effect of cold-storage temperatures upon eggs and larvæ of the Mediterranean fruit fly*—Continued

Number of days in cold storage.	Temperature of storage room.	Eggs.		Larvæ.					
				First instar.		Second instar.		Third instar.	
		Number under observation.	Number hatching after removal from storage.	Number alive.	Number dead.	Number alive.	Number dead.	Number alive.	Number dead.
	°F.								
14	33–34	1,035	0	0	76	0	245	0	49
15	33–34	746	0	0	710	0	301	3	154
16	33–34	1,058	0	1	763	0	65	0	53
17	33–34	513	0	0	521	0	45	0	134
18	33–34	1,000	0	0	514	0	46	.....	.....
19	33–34	.....	.....	0	221	0	67	0	18
8	34–36	.....	.....	.....	.....	0	11	7	170
9	34–36	.....	.....	.....	.....	0	21	1	176
10	34–36	.....	.....	0	44	0	8	5	321
11	34–36	236	0	0	192	0	60	0	225
12	34–36	.....	.....	0	74	0	138	4	399
13	34–36	241	0	.....	.....	0	84	0	436
14	34–36	.....	.....	0	111	0	19	0	354
15	34–36	.....	.....	0	42	0	6	0	158
2	36	167	131	.....	.....	120	5	242	2
3	36	281	261	166	3	261	1	260	6
4	36	419	419	127	2	245	4	180	22
5	36	433	405	288	2	473	25	256	24
6	36	365	254	75	57	334	12	158	77
7	36	184	150	28	142	147	43	62	157
8	36	454	264	1	382	0	323	33	363
9	36	858	335	1	475	0	300	2	402
10	36	301	27	0	494	0	385	0	160
11	36	652	2	0	588	0	437	0	186
12	36	728	0	0	670	0	858	0	213
13	36	534	0	0	504	0	91	0	364
14	36	463	0	0	443	0	54	1	261
15	36	568	0	0	573	0	22	1	198
16	36	480	0	.....	.....	0	38	0	251
17	36	532	0	.....	.....	.....	.....	.....	.....
3	36–40	.....	.....	.....	.....	42	2	.....	.....
4	36–40	.....	.....	.....	.....	127	46	.....	.....
5	36–40	.....	.....	.....	.....	123	3	.....	.....
6	36–40	.....	.....	.....	.....	127	25	.....	.....
7	36–40	.....	.....	.....	.....	18	94	.....	.....
8	36–40	.....	.....	.....	.....	0	13	60	258
9	36–40	136	0	.....	.....	0	25	3	112
10	36–40	128	0	.....	.....	.....	.....	.....	.....
11	36–40	125	0	0	102	0	18	0	275
12	36–40	122	0	0	23	0	12	0	256
13	36–40	.....	.....	.....	.....	0	25	0	352
14	36–40	185	0	0	32	0	275	0	522
15	36–40	.....	.....	.....	.....	0	218	0	163
16	36–40	.....	.....	0	48	0	69	0	324
17	36–40	106	0	.....	.....	0	131	.....	.....
18	36–40	.....	.....	0	118	0	18	0	97
19	36–40	210	0	.....	.....	.....	.....	.....	.....
20	36–40	.....	.....	0	16	0	64	.....	.....

TABLE I.—*Effect of cold-storage temperatures upon eggs and larvæ of the Mediterranean fruit fly*—Continued

Number of days in cold storage.	Temperature of storage room.	Eggs.		Larvæ.					
				First instar.		Second instar.		Third instar.	
		Number under observation.	Number hatching after removal from storage.	Number alive.	Number dead.	Number alive.	Number dead.	Number alive.	Number dead.
	°F.								
10	38–40			38	8	19	8	10	1
12	38–40			4	25	26	19	36	6
13	38–40			3	60	15	0		
14	38–40			0	36	17	40		
15	38–40			15	46	5	24		
16	38–40			0	99	14	148	1	25
20	38–40			0	42	0	39	4	3
23	38–40			0	43	0	84		
25	38–40			0	18	0	133	0	1
28	38–40			0	33	0	27	0	9
30	38–40			0	44				
2	40–45	12	12						
3	40–45	55	19						
4	40–45	26	0						
5	40–45	8	3						
6	40–45	16	12						
8	40–45	14	7						
9	40–45	31	17						
11	40–45	14	1						
14	40–45	31	1						
15	40–45	30	0						
17	40–45	26	6						
19	40–45	21	0			37	34	80	58
20	40–45	67	2			79	79	138	135
21	40–45	127	0			107	130	187	163
22	40–45	50	0						
23	40–45	15	0			92	97	160	220
24	40–45	21	0			68	182	125	120
25	40–45	38	0			14	281	89	88
26	40–45					30	95	106	330
28	40–45					0	9	27	268
29	40–45					1	131	57	112
31	40–45					0	161	8	201
32	40–45					0	8	4	139
33	40–45					0	290	5	318
36	40–45					0	218	7	397
37	40–45					0	345	3	393
38	40–45					0	204	7	377
39	40–45					0	42	1	385
40	40–45					0	84	0	402
41	40–45					0	112	2	330
42	40–45					0	92	0	292
44	40–45					0	39	0	200
45	40–45					0	36	1	689
46	40–45					0	23	0	476

Temperatures ranging from 32° to 33° proved equally fatal, the effect on 5,055 eggs being practically identical with that recorded for an even 32° F. Thus, no eggs hatched from batches removed between the ninth

and sixteenth days of refrigeration, although 4,475 were under observation. Only 5 eggs hatched out of 401 removed on the eighth day, and 23 out of 152 removed on the sixth day.

Temperatures ranging from 33° to 34° proved fatal after the eighth day; 45 eggs out of 300 removed on the eighth day hatched. No eggs hatched out of 6,051 removed between the ninth and eighteenth days of refrigeration.

At 34° to 36° eggs were examined only on the eleventh and thirteenth days of refrigeration. No eggs hatched out of 236 and 241 removed after these periods of refrigeration.

All the eggs subjected to a temperature of 36° were not killed until after the eleventh day of refrigeration. Out of 652 eggs removed from storage on the eleventh day, 2 hatched; and out of 301 eggs removed after 10 days, 27 hatched. No eggs hatched out of 3,305 removed after from 12 to 17 days of refrigeration. No appreciable mortality occurred at this temperature until after one week.

No eggs held at 36° to 40° were examined until the ninth day of refrigeration. Out of 1,012 eggs removed in small batches daily between the ninth and nineteenth days of refrigeration, none hatched.

Only 602 eggs were used for refrigeration at 40° to 45°. No eggs hatched after a refrigeration of 21 days. Two eggs out of 67 refrigerated for 20 days hatched on removal to the laboratory. No eggs hatched of those removed after 21 to 25 days of refrigeration.

THE LARVA

Larvæ in the third instar proved more resistant to cold than larvæ in the first and second; and all instars are generally more resistant to low temperatures than are the eggs. (See Table I.)

A temperature of 32° F. was found fatal to larvæ of the first instar after the eighth day of refrigeration; 2,558 larvæ removed after refrigeration from 9 to 14 days were found to be dead. The data in Table I show that 2 out of 845 were alive on the eighth day of refrigeration and only 11 out of 454 on the seventh day. This temperature did not appear to affect the first-stage larvæ appreciably until after the fifth day of refrigeration. Larvæ of the second instar failed to live after the ninth day, and very few lived that long; but 11 out of 473 and 20 out of 423, respectively, were alive after the eighth and ninth days of refrigeration. All of 1,868 second-instar larvæ were found dead on removal from storage after the tenth to fifteenth days of refrigeration. Only 6 out of 332 larvæ of the third instar were alive on the eleventh day of refrigeration; 626 larvæ removed after 12 to 15 days of refrigeration were found dead.

A temperature of 32° to 33° had practically the same effect upon 5,352 larvæ as did 32°.

Temperatures ranging from 33° to 34° did not prove entirely fatal to the first-instar larvæ until the seventeenth day of refrigeration; one larva out of 763 was alive on the sixteenth day. This was very exceptional and demonstrates the value of using an abundance of material and of continuing examinations after all larvæ seem to have been killed. Only 4 out of 1,446 were alive after 10 days of refrigeration; 1 out of 215 after 12 days, and 2 out of 632 after the thirteenth day of refrigeration. First-instar larvæ to the number of 1,256, removed after the seventeenth, eighteenth, and nineteenth days of refrigeration, were all dead. No second-instar larvæ subjected to 33° to 34° were found alive after the tenth day of refrigeration; 1,997 removed after 11 to 19 days of refrigeration were all dead. A few third-instar larvæ subjected to 33° to 34° lived until the fifteenth day of refrigeration, but none for a longer time. After the ninth day no larvæ were found alive, except during the examinations made after the eleventh and the fifteenth days of refrigeration, when 4 out of 126 and 3 out of 154, respectively, were found alive. A study of the data in Table I shows that a temperature of 34° to 36° had practically the same effect upon 1,615 larvæ as did that of 33° to 34°.

A temperature of 36° proved fatal to first-instar larvæ after the tenth day. After the ninth day of refrigeration 1 out of 476 was found alive. No living first-instar larvæ out of 3,272 were found alive after refrigeration from 10 to 15 days. The mortality at this temperature among first-instar larvæ became very noticeable after the sixth day of refrigeration, when 57 out of 132 larvæ were found dead. No second-instar larvæ were found alive after the eighth day of refrigeration; thus, all of 2,508 removed after refrigeration from 8 to 16 days were found dead. No third-instar larva was found alive after the ninth day of refrigeration, except on the fourteenth and fifteenth days, when 1 living larva was found out of 262 and 199 larvæ examined. After the ninth day but 2 out of 404 larvæ were found alive.

Temperature, 36° to 40° F.: No examinations were made to determine the effect of this temperature on the first-instar larvæ until after the tenth day of refrigeration. Of 339 larvæ removed after refrigeration from 11 to 20 days, none was alive. No living second-instar larva was found alive after the eighth day of refrigeration; after the seventh day 18 out of 112 were found alive. All of 868 second-instar larvæ removed after refrigeration from 8 to 20 days were dead. No living third-instar larva was found after refrigeration for 10 days, 3 out of 115 being alive after refrigeration for 9 days. All of 1,989 larvæ removed after refrigeration from 11 to 18 days were dead.

Temperature, 38° to 40° F.: All of 279 first-instar larvæ removed from storage after refrigeration from 16 to 30 days were dead, 15 out of 61 being alive after refrigeration for 15 days. No living second-stage larva was found after refrigeration from 20 to 28 days. No examina-

tions were made on the seventeenth, eighteenth, and nineteenth days; on the sixteenth day of refrigeration 14 out of 162 second-instar larvæ were alive. Third-instar larvæ were found alive after refrigeration for 20 days. No examinations were made between the twenty-first and twenty-fourth days, but no living third-instar larvæ were found during examinations of larvæ after the twenty-fifth and twenty-eighth days of refrigeration.

The warmest temperatures to which fruit flies were subjected ranged from 40° to 45°. Only larvæ of the second and third instars were used. One second-instar larva was alive on the twenty-ninth day, but no living second-instar larvæ were found thereafter, although a total of 1,658 larvæ were examined after refrigeration from 31 to 46 days. One third-instar larva was alive on the forty-fifth day. All of 476 third-instar larvæ examined on the forty-sixth day of refrigeration were dead. More data at this temperature are desirable to fix the limit safely in so far as the mature larvæ are concerned. Fruit is not, however, held at such high temperature as 40° to 45° for periods sufficiently long to kill the fruit-fly larvæ; hence, the effect of these temperatures is of far less importance than that of temperatures ranging from 32° to 40°.

CONCLUSION

The data contained in this paper show that no eggs or larvæ of the Mediterranean fruit fly survived refrigeration at 40° to 45° F. for seven weeks, at 33° to 40° for three weeks, or at 32° to 33° for two weeks. They may lead to the modification of existing quarantines and encourage the refrigeration of fruit subject to fruit-fly attack. It seems reasonable to conclude that sooner or later the certification of properly refrigerated fruit will be practicable. When an association of fruit growers or a people find it financially worth while there is no reason why they can not operate a central refrigeration plant under the supervision of an official whose reputation shall be sufficient to guarantee all fruits sent out from the plant to be absolutely free from danger as carriers of the Mediterranean fruit fly.

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BIOCHEMICAL COMPARISONS BETWEEN MATURE BEEF AND IMMATURE VEAL[1]

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INTRODUCTION

Several excellent treatises on dietetics contain statements to the effect that immature veal—i. e., veal that is about 3 weeks old or less—is unfit for human food, but these statements apparently are not based upon experimental data. At least, a search of the literature showed that too few workers have studied this subject. Certain European writers say that immature veal is bad because certain American laws forbid the sale of veal less than 3 or 4 weeks of age. Conversely, the American laws were based, to some extent, at least, upon European opinion. The desirability of further experimental work was very apparent several years ago to Drs. Melvin and Mohler, of the Bureau of Animal Industry, who started the present investigation.

The following quotations are typical of the existing literature on the subject:

Thompson (1909, p. 141):[2] Veal, especially when obtained from animals killed too young, is unusually tough, pale, dry, and indigestible; but when the animals are slaughtered at the ripe age, the meat is sometimes tender, and is regarded by many as nutritious. It differs considerably from beef in flavor, and contains more gelatin and water but less fat and protein. Veal broth is nutritious, and affords a wholesome variety in the dietary for the sick. When too much is given it may excite diarrhea. Veal is much more used for invalids in Germany than elsewhere, although it figures less conspicuously in hospital dietaries there now than formerly. Bauer declares it to be more digestible than beef, but Pavy says, referring to both veal and lamb, "they are meats that it is desirable to avoid, generally speaking, in case of dyspepsia," and this opinion is prevalent in America as well as in England.

Also (p. 420): The meat of very young animals should never be eaten, and the sale of young or "bob" veal two or three weeks old is prohibited by law. It is indigestible, innutritious, and readily decomposes.

Hutchinson states (1911, p. 67–68): Veal is believed to be somewhat difficult of digestion, a belief which is confirmed by experiment, for it required two and a half hours for its digestion, as compared with two hours for beef (Jessen). The difficulty of digesting veal is somewhat surprising, for the connective tissue, though abundant, is very easily changed into gelatin. It is believed by some that the explanation is to be found in the ease with which the fibers of veal elude the teeth on mastication.

[1] The object of the present work was to ascertain whether the flesh of calves 3 weeks of age and under is or is not fit for human food. The work was begun in the spring of 1912 at the suggestion of Dr. John R. Mohler, then Chief of the Pathological Division, Bureau of Animal Industry, and continued with little interruption up to the fall of 1914. The writer is indebted to Dr. Mohler for his very effective interest in the work and for many valuable suggestions.

[2] Bibliographic citations in parentheses refer to "Literature cited," p. 708–711.

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No experimental data on the digestibility of veal were found in the writings of Bauer (1885) and Pavy (1881), referred to by Thompson; there was nothing more than the statement that veal was not easily digested.

Although the above-mentioned work of Jessen (1883) was apparently done as accurately as the technic of that day permitted, it was far from conclusive, partly because the experiments were not numerous enough and partly because biochemical methods for accurately measuring the speed of digestion from one stage to another had not been developed. In fact, the fundamental data regarding the chemical nature of the digestive process and of the various digestion products of proteins were just then being studied. In the same volume with Jessen's work is one of the early works of Kühne and Chittenden (1883), describing the then little-known bodies resulting from the digestion of proteins.

Undoubtedly, the alleged indigestibility of veal was a belief perpetuated by repeated quotation either of experiments too old to be conclusive or of opinions expressed elsewhere.

WORK OF PREVIOUS INVESTIGATORS

With the exception of the works of Fish (1911; 1912; 1914), very little direct experimental work was found, although a careful search of the literature was made. An excellent discussion of the subject by Fish and other workers has been published by the American Veterinary Medical Association (1912). In his earlier work Fish obtained data on the amount of moisture in immature veal and in beef, also on the freezing point of the juice from the tissues and on the specific gravity of such juice. He conducted dietetic experiments in which 7 families of 20 persons of various ages received immature veal as part of their diet. The following extracts are from his reports:

> All partook of the veal and appeared to relish it. None of the families reported any disturbance of any of the bodily functions; the health was apparently normal and each family was ready to receive a portion whenever another carcass was available. (1911, p. 139.)
>
> The claim that the flesh of very young animals has a laxative effect upon human beings (Walley) has not been verified in the present experiments. (1912, p. 148.)

In a recent work Fish found that beef and immature veal digested with equal speed in pepsin-hydrochloric acid (1913, p. 64). This last observation is in accord with that of Langworthy and Holmes (unpublished), who found that both immature veal and market veal, when fed to men as part of their diet, have practically the same coefficient of digestibility as beef—i. e., 93 per cent.

Sparapani (1914) studied the toxicity, or the alleged toxicity, of fetal flesh. From his results he concluded that bovine fetal serum was less toxic than adult serum—i. e., more fetal serum was required to kill a rabbit than adult serum when injected intravenously.

EXPERIMENTAL WORK

MATERIALS

At convenient intervals a live calf, 7 days old or less, was obtained from a veterinarian in Washington, D. C., who procured the supply from farms near by. Forty-one calves were procured in this way. On 12 of these animals quantitative data were obtained; the rest of the material was used in the feeding experiments with cats. Each calf was inspected by a member of the staff of the Pathological Division. In every case, except veal sample 7, the calf purchased was found to be in good condition.

Immediately after the calf was killed, dressed, and quartered, the meat was trimmed from the bones. When the calf was intended for quantitative analytic work and for digestion experiments, care was taken to remove the muscles entire or nearly entire, so as to exclude bits of bone, tendon, etc. The whole muscles, free from adherent fat and the tough, tendinous ends, were placed in a wide-mouth 8-liter glass-stoppered bottle and kept in cold storage at or very near 1° C. (34° F.) until used.

When the calf was intended for feeding to the experimental cats, the meat was trimmed less carefully, so that adherent fat, small pieces of soft bone, etc., were included in the material stored. To this were added the liver, kidneys, spleen, lungs, and heart, all of which the cats received in their food (see p. 705). About 10 kgm. of muscle were obtained from each calf. A detailed record was made of the dates on which the calves were killed, etc., so that the age of the meat when used for the various purposes was always known.

Along with the analyses and digestions made on the veal, control determinations were made on beef. The greatest care was taken throughout the entire work to be certain that the data on beef and veal were obtained under identical conditions. Whenever a calf was killed and the veal was intended for comparative work with beef, 10 pounds of ordinary lean beef round steak were purchased in a market near by. No inquiries were made regarding the beef; it represented so much lean beef purchased at random. Soon after being brought to the laboratory the beef was carefully trimmed—i. e., fat and connective tissue were removed, leaving only the lean muscle tissue, with a few small specks of fat here and there. This was transferred to an 8-liter glass-stoppered wide-mouth bottle and kept until used in cold storage alongside the bottle containing the veal. The beef was numbered to correspond with the veal—i. e., beef sample 8 was the beef used for control work on veal sample 8.

Sometimes the comparative analyses and digestions were begun on veal and beef 1 day old—i. e., 1 day in storage—although the beef was really mature beef of unknown age. In some experiments the meats were

a month old, but in every case the age is given. Naturally, after the veal and beef had been stored for several weeks, they acquired "off odors." This was always recorded, but the meats were always used as if perfectly odorless. Veal intended for feeding to the cats was always boiled. None was rejected, no matter how unappetizing it might have been to human beings.

STANDARD SOLUTIONS AND APPARATUS

In the chemical work on the veal and beef the nitrogenous substances and the moisture content were studied. Together these constitute about 95 to 97 per cent of the weight of the meat, so that the chemical work, while not too detailed, gave information on practically all constituents except the lipins. For the large number of nitrogen determinations standard $N/5$ sulphuric acid and sodium hydroxid were used. Although all the nitrogen determinations were comparative—i. e., on veal and beef at the same time and under the same conditions—the absolute value of the standard acid was determined with the greatest care. This was done by precipitating and weighing the barium sulphate obtained from a known volume of the acid, and as an independent check on these results the acid was also standardized against pure ammonium sulphate and against pure sodium carbonate. It is perhaps true that with biological material such as meat the limit of accuracy is soon reached if ordinary care is used, and nothing is gained by taking unnecessary precautions. But because the wholesomeness of immature veal is a subject of controversy it was thought especially advisable to take too many precautions throughout the work rather than too few.

The volumetric apparatus used was standardized either by the United States Bureau of Standards or in the laboratory. A set of standardized analytic weights, a carefully calibrated Greene barometer, and a standardized thermometer from the Physikalisch-Technische Reichsanstalt (Charlottenburg, Germany), were used.

ANALYTIC DATA ON IMMATURE VEAL AND MATURE BEEF

TOTAL NITROGEN

The total nitrogen was determined on seven portions of each sample of beef and veal, of which three were made on the fresh meat, two on meat dried over sulphuric acid in vacuo at room temperature for two weeks, and two on portions dried for 12 hours at 95° C. in the hot-water oven.

No nitrogen determinations were made on veal samples 1 and 2—i. e., the first two calves—and the corresponding mature-beef samples. On veal and beef samples 3, 4, 5, 6, and 7 nitrogen determinations were made as just described. On veal and beef samples 8, 9, 10, 11, and 12

determinations were made as before, except that no portions of fresh meat were weighed for the direct determination of total nitrogen. Portions of 25 gm. each were weighed into suitable flasks and hydrolyzed by boiling with hydrochloric acid. After diluting to 250 c. c., two portions of 25 c. c. each, corresponding to 2.5 gm. of fresh meat, were pipetted into Kjeldahl flasks and the determination carried out as usual (see p. 678). In this way duplicate determinations were made on veal samples 8, 9, 10, and 11 and a single determination on sample 12. Duplicates were obtained on beef samples 8 and 11; on beef sample 10 four determinations were made and averaged, as the first two were not close enough; on beef sample 12 one determination was made. There was no beef sample 9. Veal sample 9 was compared with skim milk (skim-milk sample 2) which contained 5.29 mgm. of total nitrogen per gram of skim milk, or 0.529 per cent. Veal sample 5 was compared with beef sample 5 in some experiments and with skim-milk sample 1 in others—this contained 5.74 mgm. of total nitrogen per gram of skim milk.

All determinations of nitrogen were made by the usual Kjeldahl method, using metallic mercury, potassium sulphid, etc. Shortly after the appearance of the results of Trescot (1913), potassium sulphate was used in addition to the mercury, to assist in the oxidation. At first Congo red was used as indicator; later this was replaced by alizarin sulphonate.

The results for total nitrogen are summarized in Table I. It is apparent that the differences in nitrogen content between immature veal and mature beef are slight. The higher moisture content of the veal probably accounts for the slightly lower average figure, 3.14 per cent, as compared with 3.48 per cent for beef. The averages for the meats dried in vacuo are practically identical. For the meats dried in the hot-water oven, the average value for the veal, 14.08 per cent, is higher than that for the beef, probably because the veal dried more thoroughly—i. e., the average moisture in veal dried in vacuo was 77.08 per cent; in the hot-water oven, 77.54 per cent (see p. 683, moisture figures). The difference between the two figures for beef was not so great, the average for beef dried in vacuo being 74.18 per cent and in the hot-water oven 74.10 per cent.

TABLE I.—*Percentage of total nitrogen in meat*

Calf No.	Age of calf when killed.	Fresh.		Dried in vacuum desiccator.		Dried in hot-water oven.	
		Beef.	Veal.	Beef.	Veal.	Beef.	Veal.
	Days.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
3	7	3.45	3.33	12.67	14.03	11.78	14.65
4	5	3.49	3.18	13.56	13.86	13.65	14.03
5	6	3.51	3.24	14.62	13.04	14.26	13.37
6	5	3.60	3.00	13.23	13.26	13.42	13.60
7	5	3.59	3.40	14.40	14.41	14.47	15.12
8	3	3.53	2.95	12.60	13.55	13.52	13.74
9	7	(a)	3.12				
10	4	3.34	2.97	13.13	13.50	13.78	14.16
11	4	3.43	3.17	13.82	13.58	13.92	13.76
12	4	3.38	3.07	13.49	13.61	13.67	14.25
Average		3.48	3.14	13.50	13.65	13.61	14.08
Number of determinations averaged		24	24	18	18	18	18

a Skim-milk sample 2 was used instead of beef (see p. 695).

The figures for total nitrogen in dried meats (last four columns of Table I) were calculated back to the fresh basis for comparison with the figures obtained directly on the same samples of fresh meat, with the average results given in Table II.

TABLE II.—*Average percentage of total nitrogen in meat (dried meat calculated to fresh basis)*

Meat.	Fresh.	Dried in vacuum desiccator.	Dried in hot-water oven.
	Per cent.	*Per cent.*	*Per cent.*
Beef	3.48	3.46	3.46
Veal	3.14	3.15	3.19

It is apparent from Table II that the meats lost no nitrogen during the drying. (For the method of drying, see p. 683.) Benedict and Manning (1905, p. 312) found that "these meats [beef, chicken], therefore, after heating at 100° in a water oven lost from 4 to 7 per cent of the total nitrogen present." They quote similar observations by other investigators. What is important in this connection is not the mere loss of a small amount of nitrogen, which could be easily replaced in a diet, but the possibility that the lost nitrogen was present in the form of volatile amins, as suggested by Atwater (1895, p. 43). Some amins are very poisonous, and the presence of even small amounts of such bodies in immature veal would constitute a valid objection to its use. Although looked for, losses of nitrogen in the dried-meat samples were not observed. There may be two reasons for this: (1) The meats used were not decom-

posed, and, therefore, amins resulting from decomposition were absent; (2) the temperature inside the hot-water oven varied from 93° to 95° in winter to 95° to 97° in summer, and meat dried for 12 hours in this manner was not decomposed.

Another method of looking for toxic bodies was used, the veal being fed to cats (see p. 703).

The results for beef, summarized in Tables I and II, are practically identical with those generally obtained by other investigators. Thus, Davis and Emmett (1914, p. 449) found 3.624 per cent of nitrogen in beef dried at 100° to 105° C. for 20 hours, the result being calculated to the fresh basis. Their values for total nitrogen in beef are practically the same as those for either beef or veal in Table I. They found that there was but very slight loss, if any, on drying the meats at 100° to 105° as compared with the value found by the vacuum method. Richardson and Scherubel (1908, p. 1552) obtained the following results for total nitrogen in 13 samples of fresh lean beef: Maximum, 3.65 per cent; minimum, 3.34 per cent; average, 3.49 per cent. It is to be noticed that all the figures for fresh beef in Table I lie between this maximum and minimum, and the averages in both are practically identical. These investigators state (p. 1551) that—

> In nearly all the work on beef the muscular portion known as the "knuckle" to butchers was made use of on account of its size, uniformity in structure, and its freedom from fatty tissue. The knuckle is the group of muscles known as the Crural Triceps to anatomists and consists of the Rectus Femoris, Vastus Externus, Vastus Internus, and Anterior Gracilis. It was desired to experiment primarily upon the lean portion of beef, and fatty matter and gristle was trimmed away as far as possible in the preparation of the samples for analysis.

EXTRACTIVE NITROGEN

Portions of freshly hashed beef and veal, each weighing 100 gm. were extracted by heating in flasks with 800 c. c. of distilled water. The heating lasted one hour in a boiling water bath. After cooling and weighing the flasks, sufficient water was added to bring the final volume up to 1,000 c. c. of water plus 100 gm. of fresh meat. The total nitrogen was determined in duplicate 100 c. c. portions of the filtrates. Beginning with beef and veal samples 7, whenever meat was boiled for digestion experiments, control portions were boiled for extractive nitrogen. It is obvious that in measuring the amount of nitrogen going into solution by the digestion of meat, it was desirable to know the quantity of soluble nitrogen originally present.

In 100 c. c. of filtrate corresponding approximately to 10 gm. of meat, the extractive nitrogen actually titrated was equivalent to about 15 c. c. *N*/5 acid. In calculating the amount of nitrogen corresponding to 100 c. c. of filtrate, allowance was made for the moisture present in the meat—i. e., if the meat contained 75 per cent of water, the 100 c. c. of filtrate treated corresponded to 100/1,075 of the total extractive nitrogen

present in 100 gm. of meat. In 15 duplicate determinations on two portions of the same filtrate obtained from beef and veal samples 3 to 8, the average difference between duplicates was 0.26 c. c. *N*/5 acid; one set of duplicates on beef sample 4, in which the difference was 1.53 c. c. *N*/5 acid, was not included in this average; but the average of these two was included in the results in Table III. The data on skim milk were obtained by using 600 gm. of skim milk instead of 100 gm. of meat, making the proper calculated allowances for the water in the milk. The details of the precipitation of the casein, etc., are given on p. 692.

The results for extractive nitrogen are summarized in Table III. The last column gives the number of days that elapsed between the killing of the calf and the boiling of the meat. During this time the veal was in cold storage. This, of course, is not true of the beef. The beef when purchased was in all probability obtained from an animal killed from 8 to 18 days before. After being brought from the market, the beef was stored with the veal. While sample 3 of veal used in experiment 14 was 8 days old when boiled, the corresponding sample 3 of beef can be said to have been stored for 8 days, but its age is not known. For this reason the comparison between the two is not exact. For some purposes it might have been desirable to kill a mature animal on the premises and store the beef immediately, as was done with the veal. But the principal object was a comparison of the veal with meat as purchased in the market.

TABLE III.—*Percentage of extractive nitrogen in meat*

Sample No.	Beef.	Veal.	Experiment No.	Age of meat when boiled.
	Per cent.	*Per cent.*		*Days.*
3	[a] 0.456	[a] 0.534	14	8
4	.433	.508	15, 16	1, 9
5	.437	472	17, 18	7
5	[b] .0364	.472	19	[e] 18
6	.473	.448	20, 21, 22	2, [d] 13, [e] 21
7	.433	.646	23	3
7	.693	[f] 1.526	24	[e] 33
8	.505	.520	26	8
8	.610	.520	25	[g] 31
9	[h] .0615	.490	27	6
9	[h] .0754	.539	28	21
10	.466	.553	30	19
10	.495	.645	31	[d] 28
11	.455	.519	32	19
12	.437	.496	34	8
Average	.491	.530		
Determinations averaged	12	13		

[a] Meat hashed, kept in cold storage till next day, then boiled. All other samples hashed and boiled same day. Veal sample 5, experiment 17, was hashed and boiled the same day calf 5 was killed.
[b] Figure for extractive nitrogen in skim-milk sample 1 omitted from average.
[c] Veal had an "off odor."
[d] Beef and veal had an "off odor."
[e] Beef and veal very poor, not fit to eat.
[f] Veal sample 7, calf had white scours, figure omitted from average.
[g] Veal had no odor. Beef had slight odor of hydrogen sulphid.
[h] Figures for skim-milk sample 2 omitted from average.

With the exception of veal sample 7, all of the calves purchased were in good condition. Calf sample 7 was known to have "white scours," or diarrhea. It was plainly a sick animal and was purposely obtained. A very young kitten gained considerable weight while utilizing veal sample 7, boiled, as its sole source of nitrogen (see p. 707). The high content of extractive nitrogen in veal sample 7, experiment 23, while comparatively fresh, and its very rapid autolysis, as indicated by its appearance and still higher extractive nitrogen content in experiment 24 a month later, are very striking. The four duplicates on veal and beef samples 7 were excellent.

Hansoulle (1910, p. 122), in his report on very young veal as food, quotes Fonsny to the effect that about 60 per cent of the dry matter in meat from very young calves consists of extractives and gelatin, materials which, while digestible, are not assimilable. Hansoulle also quotes the opinions of several directors of abattoirs in Belgium and France who regard very young veal as unfit for human food, but references to experimental work are not given. After veal sample 7 had been stored for over a month, the extractive nitrogen—i. e., nitrogen soluble in water near the boiling point—amounted to 44.9 per cent of the total nitrogen in the meat. But, obviously, this was exceptional, at least for the calves used in this work. It is possible that under the conditions observed by Hansoulle the veal deteriorated rapidly and justified his strong pronouncements on the unfitness of very young veal. The data in Table III have been obtained on calves 7 days old or less when killed, the meat of which had been stored at about 34° F. (1° C.) for varying lengths of time. The differences between the figures for beef and veal are much smaller than would be expected from the statements of various writers that the elimination of excretory nitrogen in very young calves is slow. Excluding the figure for veal sample 7 in experiment 24, the average extractive-nitrogen content in fresh beef is 0.491 per cent, and for fresh veal, 0.530 per cent, with no great variations from the average. If the figure for veal sample 7 be included, the averages are 0.491 per cent for beef and 0.601 per cent for veal. The figures for beef are essentially the same as those obtained by other workers.

Richardson and Scherubel (1908, p. 1527), in their studies on cold-storage beef, extracted 100-gm. portions of fresh beef with water until 1 liter of extract was obtained from each. Determinations of nitrogen in the various forms were made on 50 c. c. portions of the extract. By adding together their figures for the amount of nitrogen present as ammonia (method 2), albumoses, and meat bases in their cold-water extract, a figure is obtained which corresponds to the figures for extractive nitrogen in Table III. The term "extractive nitrogen" is used rather loosely here, as it includes all nitrogenous substances in meat which are soluble in water near the boiling point—i. e., proteoses, peptones, amino acids, ammonia, purin bases, etc. The slight loss of ammonia due to the

coagulation of the meat and the heating in water was not determined or allowed for in the calculations; it is too small. Richardson and Scherubel (p. 1552) obtained the following averages on cold-water extracts from 13 samples of fresh beef (see p. 674): Nitrogen present as ammonia 0.010 per cent; albumoses, 0.024 per cent; meat bases, 0.071 per cent. The total, 0.405 per cent, corresponds closely to the average of 0.491 per cent for beef in Table III. It is natural that the figure in Table III should be a trifle higher, as it includes data on both fresh and cold-storage beef. The storage temperature was practically the same as that used by Richardson and Scherubel—i. e., 2° to 4° C. (36° to 39° F.). It will be noticed in Table III that while the meats were in cold storage for the periods there indicated the extractive nitrogen increased very appreciably in beef samples 7, 8, and 10 and in veal samples 9 and 10. The same probably happened in beef and veal samples 3 to 6, but data were obtained on these only when fresh.

Similar increases in extractive nitrogen were noticed by Richardson and Scherubel (1909, p. 99) in their study of the changes taking place in beef stored at 2° to 4° C. In their samples proteolysis took place more slowly than in those of Table III, probably because, as they state (p. 101), "the knuckles (weight 7 to 8 pounds) were hung in a temperature of 2° to 4° C. immediately after slaughter and were allowed to remain there during the period when analyses were made, that is for 121 days."

The meat stored for use in the present work was cut into pieces not much larger than a hen's egg of good size. Undoubtedly this treatment permitted more active autolysis and bacterial decomposition than would have taken place had the veal and beef been stored in larger masses. As previously indicated, entire muscles were dissected from the veal quarters for the sake of uniformity of composition of the muscle tissue used for analysis, etc., necessitating the storage of comparatively small pieces of meat (see p. 669).

Emmett and Grindley (1909) found that in beef stored for 22 days at 33° to 35° F. (0.5 to 2° C.) the extractive nitrogen, contrary to expectations, did not increase, but a slight increase was noticed in beef stored under the same conditions for 43 days (p. 425). It is probable that one reason for this observation is to be found in their method of preparing cold-water extracts of beef for analysis. Portions of the experimental beef weighing 30 to 35 gm. were repeatedly extracted with cold water, and the extracts after filtration were diluted to 5 liters (Grindley and Emmett, 1905, p. 663). After removing coagulable nitrogen in a 200 c. c. portion of such a filtrate, corresponding to 1.2 gm. of meat, a further partition of nitrogen was made on the very small amounts of nitrogen remaining. The unavoidable errors in analytic work become proportionately large under such conditions, and the detection of slight changes in meat stored under good conditions for short periods of time becomes difficult.

Many investigations have been made on the behavior of beef when frozen, but such results are not exactly comparable with those obtained by the foregoing investigators nor by the writer on beef stored at or near 2° C.

It is obvious that the beef and veal used in this work underwent proteolysis during the storage periods to practically the same extent. The changes that took place in the beef are entirely comparable with those observed by others in beef stored under similar conditions. The slightly higher average content of extractive nitrogen in the veal (Table III) is not regarded as physiologically significant in the present consideration of the fitness of 1-week-old veal as food. The extractives of immature veal are the same as those of mature beef (Lindsay, 1911), and the slight quantitative difference found between the 10 "bob-veal" calves and their corresponding 10 samples of lean beef (summarized in Table III) do not warrant the inference that the tissues of the very young calf are loaded with unexcreted nitrogenous waste products.

AMINO NITROGEN IN MEAT EXTRACTIVES

The hot-water extracts of beef and veal used for the determination of extractive nitrogen were also used for the determination of amino nitrogen in the nitrogenous extractives present. The figures obtained were used as blanks in those digestion experiments in which the rate of digestion was measured by the rate of formation of amino nitrogen (see p. 696). Any marked differences between the figures for beef and those for veal might have led to the detection of significant differences in their composition.

Ten c. c. of filtrate, containing the extractives from not quite 0.5 gm. of beef or veal, were introduced into the Van Slyke amino-nitrogen apparatus and the amino nitrogen determined exactly as it was determined in the digestion experiments (see p. 680). The volume of gas measured was small, ranging from 1.9 to 5 c. c. The weight of nitrogen so obtained was calculated to 1 gm. of fresh meat, and this figure was divided by the weight of extractive nitrogen in 1 gm. of meat. The results are summarized in Table IV. In experiment 27 the digestibility of veal sample 9 was compared with that of skim milk instead of beef. The amino-nitrogen determination on skim-milk sample 2 was made on 10 c. c. of diluted skim milk containing 600 gm. of skim milk diluted to 1,000 c. c. which was used for other determinations (see p. 695). In this case the amino nitrogen was derived not only from the nonprotein extractives but from the proteins as well. The amino nitrogen obtained was calculated to 1 gm. of skim milk, and this figure was divided by the weight of extractive nitrogen found in 1 gm. of skim milk by the method described on p. 695.

TABLE IV.—*Percentage of amino nitrogen in extractive nitrogen in beef and veal*

Sample No.	Experiment No.	Beef.	Veal.	Sample No.	Experiment No.	Beef.	Veal.
		Per cent.	*Per cent.*			*Per cent.*	*Per cent.*
8.....	26	27	18	11.....	32	19	16
8.....	25	27	18	12.....	34	23	19
9.....	27	[a] 60	11				
10.....	30	22	19	Average.....		24	18
10.....	31	24	24				

[a] Figure for skim-milk sample 2; not included in the average (see p. 695).

The figures for the percentage of amino nitrogen in Table IV were obtained by single determinations on each filtrate. Duplicates on veal sample 10 and skim-milk sample 2 agreed almost exactly, which is to be expected when small volumes of nitrogen gas are obtained in this determination. This, together with the comparatively large blank on the reagents, makes the experimental error in these determinations higher than in others. Nevertheless the data have been obtained on five different calves and their control samples of beef, and the uniformly higher amino-nitrogen content in the beef extractives is probably a correct indication of a slight difference between the beef and veal. The significance of this difference, if any, requires further work for its elucidation.

DISTRIBUTION OF NITROGEN IN BEEF AND VEAL HYDROLYZED BY HYDROCHLORIC ACID

HYDROLYSIS.—The beef and veal were hydrolyzed by boiling with hydrochloric acid in 300 c. c. Jena glass Erlenmeyer flasks provided with ground-in condenser tubes 100 cm. in length. Into a weighed flask 25 gm. of meat were weighed quickly to the nearest 0.1 gm. and the exact weight noted. Two such portions of beef and two of veal were weighed from large samples of the meats freshly hashed for several determinations. To each flask 175 c. c. of hydrochloric acid (1 : 1) were added. The ratio is 35 parts of 20 per cent hydrochloric acid to 1 of protein, found by Van Slyke (1912, p. 296) to hydrolyze proteins completely after boiling for 24 hours. In all the experiments except the first with beef and veal sample 8 the hydrolytic mixture was boiled for 24 hours. Beef and veal samples 8 were boiled for 24 and 48 hours, but no differences in the results were found. A small piece of broken glass added to the material in the flask prevented bumping. After boiling the required length of time the mixtures were cooled, transferred to 250 c. c. volumetric flasks, and diluted to the mark. Portions of these mixtures were used in the following determinations.

TOTAL NITROGEN.—From each of the four flasks 25 c. c. portions of the mixture, corresponding very nearly to 2.5 gm. of meat, were pipetted into Kjeldahl flasks and the total nitrogen determined. The results obtained in this way on beef and veal samples 8 to 12 have been given in Table I.

AMMONIA.—The Boussingault-Shaffer method, as described by Berg and Sherman (1905), was used for the determination of ammonia[1] in the hydrolytic mixture. The apparatus used was, in general, similar to that used by Van Slyke (1911, p. 21).

Fifty c. c. of the hydrolytic mixture, corresponding to 5 gm. of meat, were used. It was desired to know whether the general assumption that no nitrogen is carried over by the hydrochloric-acid distillate was correct or not. For this purpose the distillates from beef, whenever obtained, were transferred to the same Kjeldahl flask, while the distillates from veal were transferred to another. The total nitrogen was then estimated in the usual manner. Distillates corresponding to 25 gm. of beef and 35 gm. of immature veal yielded in both cases less than 0.2 c. c. of *N*/5 nitrogen, indicating that none was lost during the distillations.

The distillation of the ammonia was carried out as usual for one hour in every case, during which time there appeared to be no splitting off of "cleavage ammonia," as numerous tests indicated.

In the hydrolytic mixtures obtained from beef and veal the ammonia nitrogen was about 7 per cent of the total nitrogen. Because of the small amount of ammonia actually distilled, corresponding to 5 gm. of fresh meat, or about 1 gm. of protein, the unavoidable errors in the analyses are proportionately large. The differences between six duplicates on beef and veal samples 8, 10, and 11 (Table V) varied from 0.04 to 1.33 per cent of the total nitrogen; average, 0.5 per cent. An idea of the limits of accuracy of this determination may be obtained by comparing the figures for ammonia nitrogen in casein by Van Slyke (1912, p. 297), who found 10.1 and 10.27 per cent, with those by Sherman and Gettler (1913), who found 10.0 per cent.

In order to be better able to compare the results for ammonia nitrogen, etc., in beef and veal with similar results by other workers, a sample of pure casein was hydrolyzed, using 5 gm. of casein instead of 25 gm. of fresh meat. The results obtained were: On casein hydrolyzed for 24 hours, 10.04 and 10.38 per cent, and for 48 hours, 10.55 and 10.81 per cent, of the total nitrogen present as ammonia, indicating that the technic used was essentially similar to that used by the above investigators (see p. 682).

MELANIN NITROGEN.—To the mixture remaining in the distillation flask after the removal of ammonia 3 c. c. of concentrated hydrochloric acid were added, the material transferred to a 100 c. c. volumetric flask, and diluted to the mark. This was then filtered into a second clean, dry 100 c. c. flask, and the nitrogen was determined in the melanin on the filter paper, corresponding to 5 gm. of meat, by the Kjeldahl method in the usual manner. To the figure so obtained there was added the amount of melanin nitrogen occasionally obtained by filtering the hydrolytic

[1] For excellent discussions of the various methods for determining ammonia, see Smith (1913); also Shulansky and Gies (1913).

mixture before any determinations were made. The filtrate was used in the determinations of amino nitrogen.

AMINO NITROGEN.—Van Slyke's (1912) apparatus and method were used in this determination in exactly the same manner for the hydrolytic mixtures, digestion mixtures, and control determinations on the reagents, leucin and pure casein. In every case the reaction between the reagents and the solution introduced into the apparatus was allowed to go on for exactly 20 minutes. Two, and sometimes three or four, determinations of amino nitrogen were made on every sample mentioned in Table V. The distribution of nitrogen was not studied in beef and veal samples 1 to 7. Two determinations on the same solution of hydrolyzed beef or veal generally differed by 2 per cent; thus, the figures obtained for veal sample 8 were 70.2 and 72.3 per cent of the total nitrogen present in the amino form. The average of these, 71.2 per cent, is the figure recorded in Table V. The extremes in this respect were: Beef sample 11 with 71.6, 74.6, and 75.8 per cent, a difference of 4.2 per cent between the highest and lowest figures, and beef sample 12 with 74.2, 74.4, and 74.7 per cent, the difference being 0.5 per cent. When the difference of 2 per cent was obtained with the first duplicates it was believed to be due to error in procedure. Accordingly, the determinations of the next sample, veal sample 9, were made with the greatest care but with no closer results. Numerous modifications of the method were tried without the desired result. A large number of results were obtained on veal sample 9 and skim-milk sample 2, all of which were low by several per cent and have been omitted from Tables V and XII. The fact that any deviation from the procedure used for beef and veal samples 8 gave uniformly low results led to its use without modification throughout the remainder of the work.

TABLE V.—*Distribution of nitrogen in beef and veal hydrolyzed by hydrochloric acid*

[Total nitrogen = 100 per cent.]

Sample No.	Ammonia nitrogen.	Amino nitrogen.	Nonamino nitrogen, by difference.	Melanin nitrogen.	Experiment No.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	
Beef 8	7.6	70.9	20.0	1.5	26
Veal 8	7.4	71.2	19.7	1.7	
Beef 10	7.1	74.5	17.6	.8	30
Veal 10	7.1	73.1	19.0	1.0	
Beef 11	6.8	74.0	19.4	.8	32
Veal 11	7.4	70.8	20.5	1.3	
Beef 12	7.5	74.4	17.6	.5	34
Veal 12	5.6	73.5	20.3	.6	
Average:					
Beef 8 to 12	7.2	73.4	18.6	.9	.....
Veal 8 to 12	6.9	72.1	19.9	1.1	.....
Beef 1.1	6.8	75.0	17.4	.8	33
Veal 1.1	6.7	75.1	17.1	1.1	
Casein 1	10.4	71.8	16.1	1.7	29
Casein, by Van Slyke	10.1	72.1	16.1	1.8	.....

CONTROL DETERMINATIONS.—Control determinations on leucin and casein and later on tyrosin were made for the purpose of ascertaining whether errors in procedure were responsible for the unexpected differences between duplicates or whether the nature of the experimental material was such that interfering reactions made it practically impossible to obtain as close duplicates on so complex a mixture as hydrolyzed meat as can be obtained on certain pure amino acids. It is almost certain that the hydrolyzed meat contains a large variety of amino acids, some of which react quantitatively in five minutes; others require several hours; and no particular reaction time is favorable for all taken together. On this point the following quotations from the work of Van Slyke (1911) are of interest:

Time required for different classes of amino derivatives to react quantitatively. Amino groups in the α-position to carboxyl, as in the natural amino-acids, react quantitatively in 5 minutes at 20°. The group in *lysin* requires one-half hour to react completely, lysin being the only natural amino-acid which requires more than 5 minutes. *Ammonia and methylamine* require 1.5–2 hours to react quantitatively. *Urea* requires 8 hours. . . . Amino groups in *purines* and *pyrimidines* require 2–5 hours at 20° (p. 191).

Amino-acids which react abnormally with nitrous acid. Glycocoll and glycyl peptids. Glycyl-glycin, unlike the other peptids, reacts not only with its free primary amino nitrogen, but also as Fischer and Koelker have shown, with a part of the secondary nitrogen in the peptid linking. This is doubtless connected with the peculiar behavior of glycocoll itself when treated with nitrous acid. It gives off not only nitrogen, but carbon dioxide and traces of some other gas, which is not absorbed by permanganate, indicating that decompositions deeper than the deamination occur. The behavior of glycocoll and glycyl peptides can be explained in three ways: . . . (p. 197) The gas measured is about 103 per cent of the theoretical volume of nitrogen . . . (p. 199).

In the determinations on hydrolyzed meat it was observed that almost invariably the nitrogen gas measured would diminish a few tenths of a cubic centimeter in volume, if the gas were passed back into the alkaline permanganate pipette and allowed to remain there overnight. Whether this was due to the glycocoll resulting from the hydrolysis of the different proteins in the meat or to other disturbing factors can not be stated. It is probable that the secondary reactions mentioned above take place when hydrolyzed meat reacts with nitrous acid for 20 minutes, and they contribute to the difficulty of obtaining very close duplicates.

For the control determinations on leucin a sample of Kahlbaum's synthetic leucin was used. This sample was dry and contained 96.4 per cent of the theoretical total nitrogen obtained by the Kjeldahl method, indicating the presence of a non-nitrogenous impurity. Six determinations on $N/10$ leucin in 1 per cent (approximately) hydrochloric acid, made at various times throughout the work gave the following results: 95.4, 95.6, 95.3, 95.3, 96.1, and 96 per cent of the theoretical total nitrogen present as amino nitrogen; average, 95.6 per cent. One result, 94.4 per cent, obtained with exhausted permanganate in the absorption pipette,

was omitted from the average. Close duplicates on leucin and on the next control substance, casein, were obtained easily and by the identical methods that failed to produce as close duplicates on hydrolyzed meat.

The casein (casein sample 1) hydrolyzed for the control determinations was prepared in the laboratory in the usual manner, from separator skim milk. The dry protein contained 14.87 per cent of nitrogen and 0.10 per cent of ash. Although small amounts of impurities were probably present in this preparation, it compared favorably with those used by other workers. The hydrolysis of the casein, distillation of ammonia, and determination of amino nitrogen were carried out exactly as with hydrolyzed meat, except that 5 gm. of the dry casein were used instead of 25 gm. of meat. The following results were obtained: 71.37, 72.81 per cent (boiled for 24 hours), and 71.37, 71.73 per cent (boiled for 48 hours). The average of these four, 71.8 per cent, given in Table V, is very close to the figure (72.1 per cent) obtained by Van Slyke (1912, p. 297) and other investigators. The various determinations made with casein sample 1 indicate that the methods used were essentially correct and would yield close duplicates on materials to which they were applicable.

It was thought possible that the fats or their hydrolytic products might interfere with the amino-nitrogen determination, and for this reason determinations were made on beef and veal samples 1.1. These were dry, almost fat-free meat powders, prepared early in the work from beef and veal samples 1 by treating the hashed meats with alcohol and ether (see p. 685). The hydrolysis and determinations were made on these materials as usual, but no better duplicates were obtained. The figures for beef sample 1.1 were 74.1, 75.3, and 75.7 per cent; average, 75 per cent; for veal sample 1.1, 74.1, 74.1, 75.7, and 76.3 per cent; average, 75.1 per cent. The difference between the highest and the lowest figure for veal sample 1.1, 2.2 per cent, corresponds to a difference of 0.6 c. c. of nitrogen gas under the conditions of the determinations, in which the volume of gas actually measured was about 20 c. c.

A sample of tyrosin labeled "Tyrosin, pure, synthetic, Schuchardt" was also used for control determinations. It contained 1.66 per cent of moisture. Calculated to the dry basis the total nitrogen content by the Kjeldahl method was 93.5 per cent of the theoretical. The figures for amino nitrogen were 95.6, 96.0, and 95.3 per cent of the theoretical total; average, 95.6 per cent. In the first determination the gas after being measured was passed back into the absorption pipette, where it remained overnight. As usual, there was a slight diminution in volume—from 95.6 to 95.3 per cent.

It is believed that close duplicates on beef and veal have not been obtained, for reasons inherent in the material; the method used gave good results on comparatively pure leucin, casein, and tyrosin. The comparison between the amino-nitrogen content of beef and that of veal having been made under similar conditions, the data in Table V, although

possibly erroneous to the extent of 1 or 2 per cent, indicate that the differences between the mature beef and the immature veal are too slight to be significant.

NONAMINO NITROGEN.—"The difference between the Kjeldahl and NH_2 determinations gives the nonamino (NH) nitrogen. This includes one NH_2 group, that of the guanidine nucleus of arginine, which does not react with nitrous acid . . ." (Van Slyke, 1912, p. 296).

PERCENTAGE OF WATER IN BEEF AND VEAL

Two 3-gm. portions of each meat, contained in porcelain crucibles, were dried in a vacuum desiccator and two similar portions in a hot-water jacketed oven.

DRYING IN THE HOT-WATER JACKETED OVEN.—The temperature of the interior of the oven ranged from 93° to 95° C. in winter to 95° to 97° C. in summer. The samples were transferred to the oven immediately after being weighed and were dried for 12 hours. A slow stream of clean dry air was passed through the drying chamber for several hours during the drying period, after which the crucibles were transferred to a desiccator, cooled, and weighed.

DRYING IN THE VACUUM DESICCATOR.—The samples of hashed beef and veal were transferred to a Hempel's desiccator; this was evacuated to about 85 mm. of mercury and the drying allowed to take place for two weeks at room temperature. During this time the sulphuric acid was changed once, and the desiccator was evacuated several times.

The dried samples, after being weighed, were transferred at convenient times to Kjeldahl flasks for nitrogen determinations except beef and veal samples 1 and 2. On these ash was determined by igniting the dried material. The results were: Beef sample 1, 1.16 per cent; beef sample 2, 1.10 per cent; veal sample 1, 1.14 per cent; veal sample 2, 1.14 per cent of ash. Calf 1 was 5 days old when killed; calf 2, 3 days. The ages of the others have been given in Table I. The results for water in beef and veal are summarized in Table VI.

TABLE VI.—*Percentage of water in beef and veal*

Sample No.	Dried in vacuum desiccator 2 weeks at room temperature.		Dried in water-jacketed oven 12 hours at 95± 2° C.	
	Beef.	Veal.	Beef.	Veal.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	73.25	76.83	73.58	77.10
2	73.59	78.74	74.03	79.83
3	72.98	77.13	71.49	76.37
4	75.28	77.39	74.84	77.39
5	76.46	74.38	75.01	75.79
6	72.69	76.12	73.63	77.60
7	75.13	78.01	75.12	77.98
8	72.54	77.76	71.35	77.93
10	74.48	77.42	75.45	78.38
11	75.11	76.86	75.99	77.05
12	74.43	77.20	74.66	77.54
Average of 22 determinations	74.18	77.08	74.10	77.54

Although care was taken to insure as uniform sampling as possible, the differences between duplicates varied from 0 per cent to 4.70 per cent—i. e., the figures for veal sample 11 were 77.05 and 77.05 per cent; for beef sample 8, 69 and 73.70 per cent. In every case the average of the dulpicates is given in the table. The average of the 44 differences (there were 44 duplicates) was 0.92 per cent. Although theoretically simple, the determination of water in such material as meat is practically very difficult.[1] The results for beef and for veal are strictly comparable in so far as both sets were obtained under the same conditions, but they are not exact in the absolute sense. Had the samples been heated for more than 12 hours in the hot-water oven, the "moisture content" would have been higher, partly because more water would be driven off, and partly because other substances would volatilize, decompositions would begin, etc. Apparently, under the conditions of the determinations, errors which result from heating meat over 100° C. for long periods of time were obviated (Davis and Emmett, 1914).

The figures in Table VI for beef are similar to those obtained by other workers. Richardson and Scherubel (1908, p. 1527, 1552) found an average of 76.35 per cent of moisture in beef which had been dried to constant weight at 100° to 105° C. Grindley and Emmett (1905, p. 659) found 75.46 per cent of moisture in beef dried in a hot-water oven for a length of time not stated.

Obviously, the claim that immature veal ("bob veal") is more watery than beef finds little support in the data obtained, because the difference between the averages, about 3 per cent, is physiologically of no importance.

COMPARATIVE DIGESTIBILITY OF MATURE BEEF AND IMMATURE VEAL IN VITRO

In the following comparative measurements of the speed of proteolysis of beef and veal, an attempt was made to ascertain whether immature veal is more resistant to pepsin and trypsin than beef, as sometimes stated. Three separate methods were used, each of which has its advantages, disadvantages, and errors. In the first method the undigested meat was filtered at the end of the digestion period, dried, and weighed. In the second, nitrogen was estimated in portions of the digestive fluid from time to time, thereby giving an indication of the rate at which nitrogenous substances were going into solution. In the third, the rate of formation of amino nitrogen was estimated in portions of the digestive fluid, indicating the rate at which the amino-nitrogen groups interlocked in the polypeptids present were opened or separated by the trypsin and alkali.[2]

[1] For a discussion of the errors entering into this determination, see Benedict and Manning (1905).

[2] For discussions of the earlier work on artificial digestion, see Grindley, Mojonnier, and Porter (1907, p. 61), and Berg (1909).

SOLUTIONS.—Digestions were made in 0.2 per cent hydrochloric-acid and in 0.5 per cent sodium-carbonate solutions.

ENZYM PREPARATIONS.—The following preparations, all in powder form, were used:

Pepsin 1: A 100-gm. bottle of pepsin (1:3,000), Parke, Davis & Co.; purchased about May, 1912.

Pancreatin 1: A 1-ounce bottle of pancreatin (Parke, Davis & Co.); an old preparation.

Trypsin 1: A 1-ounce bottle of trypsin (Merck); purchased in September, 1912.

Trypsin 2: A 200-gm. bottle of trypsin sicc. (Greubler); imported about March, 1912.

Trypsin 3: A 50-gm. bottle of trypsin (Merck); purchased in August, 1913.

In every case the unopened bottle of enzym preparation was used. Portions were transferred to weighing bottles and dried for several days in desiccators until the loss in weight was slight. The bottles were then stoppered. The day before being used the enzym preparations were dried in a desiccator and portions weighed as needed.

In order to correct the digestion data for nitrogen introduced in the form of enzym, their nitrogen contents were determined. The results are summarized in Table VII. The methods used were similar to those employed throughout the work.

TABLE VII.—*Quantity of N/5 nitrogen per gram of dry enzym preparation*

Preparation.	Total nitrogen.	Ammonia nitrogen.	Amino nitrogen.
	C. c.	*C. c.*	*C. c.*
Pepsin 1	51.1	.....	.....
Pancreatin 1	40.9	.....	.....
Trypsin 1	47.3	None.	.....
Trypsin 2	70.1	62.5	2.0
Trypsin 3	47.0	1.0	22.8

All of the digestion experiments were begun with freshly hashed beef or immature veal, except experiments 5 to 8, in which powdered meats beef sample 1.1 and veal sample 1.1, were used. These were prepared as follows:

Veal sample 1.1: Seven kgm. of veal sample 1 were hashed and transferred to two 8-liter wide-mouth bottles. Seven liters of 50 per cent alcohol were added and the mixture well stirred. After 24 hours the 50 per cent alcohol was strained off through cheesecloth and replaced with an equal volume of 75 per cent and the next day with 95 per cent alcohol This was followed by two treatments with 2 liters of absolute ether. The ether was removed by straining through cheesecloth and squeezing

the material, after which most of the ether was removed by exposing the veal to the air in large crystallizing dishes. The veal was then heated in the hot-water oven at 85° C. (flame out) for two hours, and bottled.

Beef sample 1.1: Fourteen hundred grams of beef sample 1 were treated with alcohol and ether in exactly the same way as veal sample 1.1, using 1,400 c. c. of alcohol, etc.

When portions of these powdered-meat preparations were weighed for digestions, portions were also weighed for moisture determinations, so that the final weights were based on the dry material. Total nitrogen per gram of beef sample 1.1, 57.2 c. c. *N*/5 nitrogen; per gram of veal sample 1.1, 57.8 c. c. *N*/5 nitrogen. Other analytic data are given in Table V.

FIRST METHOD: WEIGHING THE UNDIGESTED MEAT RESIDUES

Portions of 5 gm. each of the raw hashed beef and veal were weighed into 200 c. c. Erlenmeyer flasks. After adding 40 c. c. of water to each flask and stirring, the flasks were kept in a boiling-water bath for 1 hour. They were then cooled, weighed, and the evaporated water replaced. To each flask 50 c. c. of 0.4 per cent hydrochloric acid were added, followed shortly afterwards by the addition of 10 c. c. of the pepsin solution. Three flasks containing beef and three containing veal were generally used in a single experiment (see Table VIII). In the controls on the acid 10 c. c. of water instead of the pepsin solution were added.

The digestion was considered to have begun when the pepsin was added. During the digestion period the flasks were rotated occasionally, so as to mix the contents. When the digestion period had ended, the filtration of the residue, consisting of undigested meat, fat, etc., was begun. For this purpose loose-textured filter papers (Schleicher & Schull's No. 589, white band, 15 cm.) were used. These papers, contained in weighing bottles, had previously been dried for several hours at 95° C. in the hot-water oven until the change in weight after a second drying was slight. Drying such papers to absolutely constant weight was as difficult as drying meat to constant weight without decomposition or oxidation.

It is at this point that the worker loses control over the method. When filtration was rapid, which sometimes happened, the separation of undigested meat from the pepsin-hydrochloric-acid solution ended the digestion period quite sharply, so far as the residue was concerned. But, as was generally the case, filtration was slow because the residue was gelatinous and clogged the filter, and it was not possible to end the digestion period shortly after filtration was begun because digestion continued as long as the pepsin-hydrochloric-acid solution was in contact with undigested meat. Fortunately, the digestive process becomes slow as the meat approaches complete digestion, so that the error from this source probably amounts to less than 10 per cent of the correct result.

When filtration was complete or nearly so, the residues were washed with water, transferred with the paper to the corresponding weighing bottles, and dried to approximately constant weight at 95° C. in the hot-water oven. From the data for moisture, the original 5-gm. portions of fresh meat were calculated to the dry weight. The weight of the dry, undigested residue divided by the corresponding weight of dry meat gave the percentage of beef or veal present as undigested residue (see "Percentages of meat digested," p. 700).

ACID PROTEINATE.—The value of the determination of acid proteinate in digestion mixtures has been pointed out by Gies (Hawk and Gies, 1902). The first step in the

digestion of a protein by pepsin-hydrochloricacid solution is the combination of the protein and the acid to form a class of substances known as acid proteinates. These are soluble in dilute acids and alkalies, but are insoluble in water.

The filtrates obtained at the end of the digestion period contained (1) the acid proteinates and (2) the next cleavage products of the acid proteinate, the proteoses and peptones. A measured amount of filtrate, generally between 50 and 80 c. c., taken before the washing of the residue was begun, was nearly neutralized with *N*/5 sodium hydroxid. The exact amount added varied in the different experiments; calculated to 100 c. c. of filtrate it varied around 21 c. c. The 100 c. c. of 0.2 per cent hydrochloric acid in which the digestions were made were equivalent to 28 c. c. of approximately *N*/5 sodium hydroxid. The addition of alkali was stopped when a flocculent precipitate of acid proteinate was thrown down. The mixture was then rapidly brought to a boil and filtered on a weighed paper. This was dried along with the undigested residues, and the results calculated in the same way.

The difficulties involved in promptly checking the action of the pepsin at the end of the digestion period were very apparent to Grindley, Mojonnier, and Porter (1907, p. 68), who after many trials found that the addition of formaldehyde solution to a digestion mixture brought the digestion to a close. Differences in length of time required for filtration will not then involve the error previously mentioned. This method, however, is not the only one. By using small amounts of pepsin the digestion period may be made long; and then it makes little difference whether a particular mixture requires a few more or a few less hours to filter completely. An objection to this procedure is that the acid alone in the control may digest as much as the acid plus the small amount of pepsin, and the action of the pepsin under such conditions can not be measured with certainty. Further, the amount of pepsin must not be large enough to permit the digestive processes to go to completion, for the undigested residue then obtained represents material not digestible under the conditions, and no information is obtained regarding the rate at which digestion took place. If allowed time enough, both a fast horse and a slow horse will be found at the same place at the end of a race. In experiment 13, Table VIII, the undigested residues obtained after long digestion with fairly large amounts of pepsin represented the amount of meat constituents not digestible by the pepsin-hydrochloric-acid solution.

No information as to whether the beef or the veal digested faster could be obtained from such data. That the residues in this experiment were almost certainly fat is indicated by the results of Table IX, with which experiment 13 is comparable because the concentration of pepsin was the same in both—i. e., 10 mgm. to 100 c. c. of 0.2 per cent hydrochloric acid. Under these conditions practically all of the nitrogen in the beef and veal went into solution in 24 hours, leaving the fat, which is not digested by pepsin-hydrochloric-acid solution. Fat determinations were not made. According to Fish (1911, p. 132), beef contains more fat than ordinary veal. This is probably still more true of immature veal. The larger residues from beef in experiment 13 are in accord with the data of Fish.

TABLE VIII.—*Comparative digestibility of mature beef and immature veal in pepsin-hydrochloric-acid solution*

BEEF AND VEAL SAMPLES I

Experiment No.	Digestion period.	Filtrate neutralized after—	Pepsin I.	Percentage of beef present as—		Percentage of veal present as—		Temperature.
				Undigested residue.	Acid proteinate.	Undigested residue.	Acid proteinate.	
			Mgm.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	°C.
1	8 days	4 hours	.....	79	4	74	5	Room.
			.....	79	8	73	5	
			0.01	64	16	63	9	
			.01	63	12	60	10	
2	8 days	4 hours	.10	22	31	29	21	Do.
			.10	25	29	26	22	
3	1½ hours	½ hour	.....	84	4	85	4	40
			10.0	43	(a)	52	5	
			10.0	43	11	54	6	
4[b]	3 hours	½ hour	.....	66	22	45	12	40
			10.0	30	14	34	7	
			10.0	24	16	27	8	

BEEF AND VEAL SAMPLES I.I

5[c]	46 days	24 hours	.....	12	11	20	16	Room.
			.01	11	37	37	4	
			.01	8	23	43	34	
6	10 days	10 hours	.....	82	11	78	10	Do.
			.10	15	38	42	34	
			.10	17	38	34	35	
7	4 hours	1 hour	.....	88	10	94	6	Do.
			10.0	11	25	33	21	
			10.0	11	26	33	21	
8	3 hours		.....	87		85		40
			10.0	25		27		
			10.0	25		28		

BEEF AND VEAL SAMPLES 2

9	4 hours	½ hour	.....	84	6	79	4	40
			10.0	27	7	21	7	
			10.0	30	7	21	6	
10	4 hours		.....	78		73		40
			10.0	42		32		
			10.0	44		29		
11	4 hours	½ hour	.....	76	11	77	3	40
			10.0	36	9	25	6	
			10.0	44	9	28	6	
12	4 hours		.....	80		77		40
			10.0	53		32		
			10.0	50		33		
13	23 days	4 hours	.....	74	14	73	9	Room.
			10.0	20	0	15	0	
			10.0	15	0	12	0	
			10.0	20	0	12	0	
			10.0	23	0	11	0	
			10.0	19	0	.....	.....	

[a] Determination lost.

[b] The flasks containing the 5-gm. portions of hashed beef and veal were kept in cold storage at 2° C. for three weeks, during which time autolysis went on. This probably accounts for the small residues in the blanks, which contained hydrochloric acid but no pepsin. While in cold storage the flasks contained nothing but the meat.

[c] Experiment 5 is to be rejected. The continued action of molds during the digestion period invalidated the results.

A second method of checking the action of the pepsin-hydrocloric-acid solution used in experiments 8, 10, and 12 involved nothing more than the neutralization of the digestion mixture at the end of the desired time. Pepsin digests in the presence of free acid; it does not act in neutral solutions with any appreciable speed. Thus in experiment 10, and in experiment 12, which was a repetition of experiment 10, exactly four hours after the digestion was begun by adding the pepsin solution to 5-gm. portions of meat suspended in 100 c. c. portions of 0.2 per cent hydrochloric acid, the entire mixture was neutralized by the addition of 21 to 25 c. c. of *N*/5 sodium hydroxid. This checked the peptic action at once, but also precipitated the acid proteinate. The mixture was then quickly brought to a boil, after which filtration, whether fast or slow, may be continued at the convenience of the worker. Obviously the residue in this case does not give as detailed information as that obtained by filtration of undigested residue and precipitation of acid proteinate in the filtrate. In experiments 10 and 12 the veal digested a little faster than the beef.

In experiments 5 to 8, practically fat-free beef and veal, prepared as described on page 685, were used. The object was to eliminate the error due to the fat, which, when present, is weighed with the undigested protein. One-gm. portions of the dry powders were used instead of the 5-gm. portions of fresh meat. Otherwise the procedure was the same as in the other experiments, except that, in so far as the proteins present had already been coagulated by exposure to alcohol, ether, and a temperature of 85° C., heating the mixture of meat powder and water in a boiling-water bath was omitted. The results in experiment 5 were invalidated by molds. In experiments 6 to 8 the results indicate a slightly more rapid digestion of beef sample 1.1.

The most interesting results in Table VIII are those of experiments 1, 2, and 6. In experiment 1 so minute a quantity of pepsin as 0.01 mgm. in 100 c. c. of 0.2 per cent hydrochloric acid exerted an equally distinct digestive action on both the beef and veal. With 0.1 mgm. of pepsin the digestion was unmistakable, indicating that in these particular cases the immature veal was as susceptible to the action of minute amounts of pepsin as the mature beef. To ascertain whether this was true or not was the object of experiments 1 and 2.

It will be noticed that in the experiments summarized in the table the amounts of pepsin used varied from 0.01 mgm. to 1,000 times this amount—i. e., 10.0 mgm. A wide range of enzym concentration in such work is not only desirable but almost necessary. What is true at one concentration of enzym may not be true at another very different one. Thus, Berg and Gies (1907) found that in acetic acid fibrin would digest very slowly when the amount of pepsin present was comparatively small, but in the presence of large amounts of this enzym digestion proceeded with a wholly unexpected speed.

A comparison of the results for beef in Table VIII with some of the data obtained by Grindley, Mojonnier, and Porter (1907, p. 66) in their artificial-digestion experiments can not very well be made. These investigators used 250 mgm. of pepsin per 100 c. c. of 0.33 per cent hydrochloric acid. The kind of pepsin preparation used was not stated, but, assuming it to be the usual 1 to 3,000 product, their digestion mixtures contained 25 times as much pepsin as the strongest digestion mixtures mentioned in Tables VIII or IX. Their conditions of comparatively high pepsin and high acid concentration probably were not favorable for the detection of small differences in digestibility, although these conditions may have been desirable for other reasons.

Perhaps the only work with which the data of Table VIII can be compared are the recent results obtained by Fish (1914) on the comparative digestibility of beef, market veal, and immature veal. In the absence of a statement pertaining to the treatment of the meats, the inference may perhaps be drawn that the digestion experiments were made on raw meats. Otherwise, the general method and conditions of Fish's digestion experiments were similar to those in experiments 1 to 13. Samples from 22 immature

veal calves were compared with an almost equal number of samples of market veal and beef, using "3.35 milligrams of scale pepsin" in 100 c. c. of 0.2 per cent hydrochloric acid. Fish (p. 52–53) concludes this part of the work with the following statement:

The results show that, as regards the averages, the differences in the digestibility of the tissues of bob veal and market veal are so slight as to be negligible; but such as they are, they are slightly in favor of the bob veal as a whole. The differences between the beef and veal is [sic] more noticeable, but the apparent greater digestibility of the veal may be due in part to the fact that as a rule there is a slightly smaller percentage of water present in the beef as well as a somewhat greater amount of connective tissue. As the greatest difference shown by the averages is but 3 per cent under the conditions of the experiments, it would indicate no serious difficulties in the digestibility of any of the material.

A redeeming feature of the method used in experiments 1 to 13 is its simplicity, both in the technic used and the equipment required. That the results obtained are substantially correct is indicated by the fact that repetitions of the measurements, using different methods, involving different errors, yielded similar results.

SECOND METHOD: MEASURING THE RATE OF FORMATION OF PROTROSE, PEPTONE, AND AMINO-ACID NITROGEN

Into each of two 2-liter Jena Erlenmeyer flasks 100 gm. of freshly hashed beef were weighed to the 0.1 gm. Similar portions of veal were weighed into two similar flasks. After adding 750 c. c. of water to each flask and stirring, the flasks were kept in a boiling-water bath for one hour. They were then cooled, weighed, and the evaporated water replaced. The stoppered flasks remained in cold storage overnight. Two of these, one of beef and one of veal, were used for the determination of extractive nitrogen as already described on p. 673. The next morning the flask containing the beef and the flask containing the veal for the digestion experiment were quickly warmed to 40° C. The dry, powdered enzym was then added, followed by 1 liter of 0.4 per cent hydrochloric acid or of 1 per cent sodium-carbonate solution. Water was then added to bring the final volume up to 2,000 c. c. In this way every digestion experiment was begun with 100 gm. of beef or veal, plus 2,000 c. c. of 0.2 per cent hydrochloric acid when pepsin was used (see Table IX), or 2,000 c. c. of 0.5 per cent sodium carbonate when trypsin was used (see Table X). During the course of the digestion the flasks were kept in a 40° C. water bath, except when they were removed to mix their contents or to take samples for analysis. The treatment of the digestion mixtures containing pepsin-hydrochloric-acid solution and those containing trypsin-sodium carbonate solution will be described separately.

DIGESTION IN PEPSIN-HYDROCHLORIC-ACID SOLUTION.—During the earlier part of the experiment the contents of the flasks were mixed about every 15 minutes. Later, when most of the meat had gone into solution, the mixing was done at longer intervals, but always the same for both flasks. In the experiments summarized in Table IX the rate of digestion was measured at the time intervals there indicated by removing 100 c. c. portions of supernatant digestion fluid and determining in this the amount of nitrogen present as acid proteinate, proteoses, and peptones. By difference the nitrogen in the undigested residue could be obtained. If, for example, it was desired to obtain data on veal for one hour's digestion, the veal mixture was well mixed 45 minutes after the digestion was begun and was allowed to remain in the water bath for 10 minutes, in order to allow meat particles to settle to the bottom of the flask. The flask was then removed from the bath, and with a calibrated 100 c. c. pipette 100 c. c. of the supernatant suspension was transferred to a 200 c. c. Erlenmeyer flask. Exactly 60 minutes after the digestion began, the action of the pepsin-hydrochloric-acid solution was stopped by nearly neutralizing the contents of the 200 c. c. Erlenmeyer flask by the addition of $N/5$ sodium hydroxid and bringing it to a boil by heating directly over a Bunsen burner. The flask containing the digestion mixture was

replaced in the bath. The quantities of *N*/5 sodium hydroxid used varied from 18 to 29 c. c. The neutralization is satisfactory when a flocculent precipitate appears. In the same way 100 c. c. of the digestion fluid from the beef mixture were removed and neutralized 60 minutes after starting the beef digestion.

In this way portions of the digestion mixtures of beef and veal were removed for neutralization on the minute, at intervals of 1, 2, 4, 7, and 24 hours. Fifteen minutes before neutralization the flask contents were mixed and allowed to stand for 10 minutes. A 100 c. c. portion was then removed from the bulk of the digestion mixture 5 minutes before neutralization.

The precipitated acid proteinate was filtered, washed, and nitrogen was determined by the Kjeldahl method. The results obtained are given in Table IX under the heading "Quantity of *N*/5 acid-proteinate nitrogen."

The filtrate was transferred to a Kjeldahl flask and the total nitrogen determined. This filtrate contained nitrogen derived from (1) the proteoses and peptones formed by the digestion of the meat, (2) the extractives present before digestion began, and (3) the pepsin. The figure for total nitrogen obtained on the filtrate is the sum of these three. The data recorded in Table IX under the heading "Quantity of *N*/5 proteose and peptone nitrogen" are the figures actually obtained and corrected for the sum of the extractive and pepsin nitrogen. Thus, in experiment 14 the results obtained for one hour's digestion of beef sample 3 were, for the precipitated acid proteinate, 2.7 c. c. of *N*/5 nitrogen; for the filtrate, 23.8 c. c. From this latter figure there was subtracted 8.0 c. c., this being the sum of the extractive nitrogen in that sample of beef at that time, and the nitrogen present in the pepsin added. The method of determining extractive nitrogen is described on page 673.

During the digestion the water contained in the meat is liberated and dilutes the digestion fluid to a slight extent. No correction for this was made, except in those particular cases where the correction is indicated.

The "theoretical maximum" for proteose and peptone nitrogen in 100 c. c. of digestion fluid was calculated in the following manner: The sum of the total nitrogen in 100 gm. of fresh meat plus the pepsin nitrogen was divided by the volume of the digestion fluid at complete digestion—i. e., 2,000 c. c. plus the volume of water in the 100 gm. of meat.

By the term "Age of meat, days," at the bottom of Table IX is meant the number of days the meat was in cold storage before being boiled. Thus, in experiment 21 beef sample 6 and veal sample 6 were hashed and boiled after 13 days in cold storage, and on the next day digestion was begun. These figures do not refer to the age of the calf when killed, this having been given in Table I.

It will be noticed that the theoretical maximum for proteose and peptone nitrogen is approximately 50 c. c. of *N*/5 nitrogen in nearly all the experiments. In order to obtain the percentage of nitrogen present as proteoses and peptones at any time, it is only necessary to multiply the corresponding figure by 2. Thus, in experiment 19, at the end of seven hours approximately 82 per cent of the veal (41.0÷48.0) had been transformed into proteoses and peptones. It is obvious that both the beef and the veal were digested with practically the same speed and that at the end of 24 hours the transformation into proteoses and peptones was complete.

For practical purposes the digestive process may here be regarded as taking place in two stages: (1) The transformation of the native meat proteins to acid proteinate by combination with the hydrochloric acid, and (2) the cleavage of the acid proteinate into the smaller molecules of proteoses and peptones.

The data in Table IX indicate that both processes took place with equal speed in the beef and veal.

The undigested residues weighed in experiment 13, Table VIII, probably contained very little nitrogen. The concentration of pepsin in experiments 9 to 13 was the same

as in the experiments in Table IX. By comparing the results of experiment 13 with those of experiment 14, for example, it will be apparent that the undigested residues in experiment 13 give an imperfect idea of the amount of indigestible protein present in beef and veal; according to the data of Table IX practically all of the nitrogen was in soluble form at the end of 24 hours.

The conditions of the experiments in Table IX were as follows: In each experiment the digestion mixture consisted of 100 gm. of meat plus 2,000 c. c. of 0.2 per cent hydrochloric acid plus 200 mgm. of pepsin 1. For nitrogen determinations 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat, were used.

TABLE IX.—*Rate of formation of proteoses and peptones in pepsin hydrochloric-acid solution*

QUANTITY (IN CUBIC CENTIMETERS) OF N/5 PROTEOSE AND PEPTONE NITROGEN

Digestion period.	Experiment No. —									
	14		17		19		21		23	
	Beef sample 3.	Veal sample 5.	Beef sample 5.	Veal sample 5.	Skim milk sample 1.	Veal sample 5.	Beef sample 6.	Veal sample 6.	Beef sample 7.	Veal sample 7.
Hours.										
1	15.8	10.3	15.6	16.8	27.7	12.6	15.7	12.1	17.1	11.6
2	24.7	20.5	26.8	26.6	37.2	23.4	23.4	19.3	27.9	18.5
4	33.8	31.1	37.8	36.3	43.8	34.6	33.1	29.6	37.9	29.0
7	41.8	39.5	45.3	41.4	46.2	41.0	41.0	37.8	44.2	36.7
24	50.7	47.1	52.3	46.7	50.4	46.6	52.8	(a)	52.9	45.5
Theoretical maximum	51.8	48.3	53.1	48.0	54.8	48.0	53.8	43.8	54.2	47.3
Extractive nitrogen	7.5	8.9	7.2	7.8	3.7	7.8	8.1	7.7	7.5	11.1
Pepsin nitrogen	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5

QUANTITY (IN CUBIC CENTIMETERS) OF N/5 ACID PROTEINATE NITROGEN

Hours.										
1	2.7	1.5	4.6	4.2	26.0	2.4	2.8	1.6	3.3	1.5
2	3.9	2.8	4.8	4.9	17.3	4.5	4.7	3.2	5.2	3.7
4	3.4	3.6	5.0	4.6	10.8	4.6	5.4	4.3	4.9	4.0
7	3.3	4.3	3.8	5.1	8.2	5.6	5.2	5.9	4.1	4.2
24	3.0	4.0	3.3	4.2	4.3	4.9	3.7	4.5	2.7	4.0
Age of meat, days	8	8	0	0	18	18	13	13	3	3

(a) Determination lost. Result obtained at 53 hours (47.1 c. c.) is probably incorrect, being larger than the theoretical maximum for that mixture.

The results of the experiments in Table IX can be plotted, and curves, of which the following are typical, obtained (fig. 1).

After several comparisons of veal with beef showed no appreciable differences between the two as regards their behavior in pepsin hydrochloric acid or in trypsin sodium carbonate, it was desirable to compare the veal with some other protein material in order to be certain that the method used would detect a difference in the

rate of digestion when such a difference existed. Accordingly, in experiment 19, veal sample 5 was compared with a sample of raw skim milk obtained in the fresh condition from the Dairy Division, Bureau of Animal Industry. Instead of 100 gm. of beef, 600 gm. of the skim milk were transferred to a 2-liter Erlenmeyer flask. The specific gravity of skim-milk sample 1 was 1.0352 at 26° C., and, hence, the volume of the 600 gm. was 600/1.0352, or 579.2 c. c. This was regarded as if it were 100 gm. of beef plus 479 c. c. of water. To this amount, 316 c. c. of water were added, the milk being kept in a boiling-water bath for five minutes. It was kept in cold storage overnight with veal sample 5; the next morning it was treated in the usual way along with this sample. At the beginning of the digestion the volume of the skim-milk digestion mixture was 2,096 c. c., which is practically the volume of the meat mixtures—i. e., 2,000 c. c. plus the volume of 100 gm. of meat, which lies between 75 and 100 c. c. A similar sample of skim milk in 0.2 per cent hydrochloric acid was used for the determination of extractive nitrogen. Skim-milk sample 1 con-

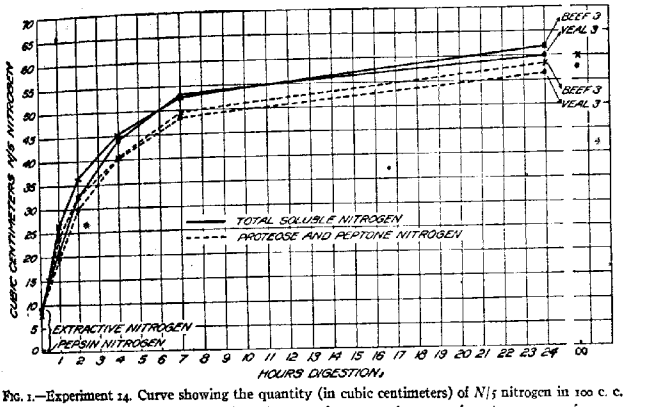

FIG. 1.—Experiment 14. Curve showing the quantity (in cubic centimeters) of $N/5$ nitrogen in 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat; used 100 gm. of meat, 2,000 c. c. of 0.2 per cent hydrochloric acid, and 200 mgm. of pepsin 1.

tained 2.05 c. c. of $N/5$ nitrogen per gram, or 0.574 per cent. The extractive nitrogen was 6.3 per cent of the total nitrogen.

In precipitating the undigested proteins and the acid proteinate by neutralization and heat, care was taken to test the filtrates with acid and alkali, in order to be certain that precipitable protein was not present in any of the filtrates. The complete precipitation, though troublesome, was not difficult. The precipitates, containing both undigested proteins and acid proteinate, were determined for nitrogen by the Kjeldahl method in the usual manner and the results recorded under the heading "Quantity (in cubic centimeters) of $N/5$ acid proteinate nitrogen." The figures for proteose and peptone nitrogen obtained from the filtrates indicate that this transformation was more rapid in the skim milk than in the veal. This is, of course, easily accounted for by the fact that the skim-milk proteins were in solution or suspension at the beginning of the digestion, while the veal particles took time to go into solution.

DIGESTION IN TRYPSIN SODIUM CARBONATE SOLUTION.—In general, these experiments were carried out in exactly the same way as the digestions in pepsin hydrochloric acid solution. Dry, powdered trypsin preparations were used. Portions of these were weighed and transferred to the digestion mixtures in the same way as the pepsin. Instead of 1 liter of 0.4 per cent hydrochloric acid, the same volume of 1 per cent sodium carbonate was added. The digestions in experiments 15 to 34 (Tables X and XI)

were all made in 0.5 per cent sodium carbonate. Although trypsin 1 and trypsin 3 had the same total nitrogen contents (see Table VII), trypsin 1 was the more active preparation. This is evident from the fact that in experiments 18, 20, and 22 (Table X) digestion had proceeded as far in seven hours as in experiments 32 and 34 at the end of six hours, although in the latter experiments twice the weight of trypsin was used.

The 100 c. c. portions of digestion fluid were neutralized with 24.5 c. c. of 2 N 2/5 sulphuric acid, the exact strength of which was N 2/5 × 0.98. This was sufficient to neutralize the sodium carbonate present and leave about 0.5 c. c. of the acid in excess, preventing the escape of ammonia when the mixture was brought to a boil. The filtration and determination of total nitrogen in the precipitated alkali proteinate and in the filtrate were carried out as described in the acid digestions.

It is to be noted that, while small amounts of pepsin in hydrochloric acid will rapidly digest meat proteins to the proteose and peptone stage but no further, trypsin, although much slower in its action, will further split the meat proteins into amino acids. This is the reason for the data under "Quantity of N/5 proteose, peptone, and amino-acid nitrogen" in Tables X and XI. The statement of results in Table X is, in general, similar to that in Table IX.

The conditions of experiments 15 to 24 were as follows: In each experiment the digestion mixture consisted of 100 gm. of meat plus 2,000 c. c. of 0.5 per cent sodium-carbonate solution plus 2.000 gm. of trypsin 1; except experiment 15, in which 2,000 gm. of pancreatin 1 was used. For nitrogen determinations, 100 c. c. of digestion fluid, equivalent to approximately 5 gm. of meat, were used.

TABLE X.—*Rate of formation of proteoses, peptones, and amino acids in trypsin-sodium-carbonate solution*

QUANTITY (IN CUBIC CENTIMETERS) OF N/5 PROTEOSE, PEPTONE, AND AMINO-ACID NITROGEN

Digestion period.	Experiment No.											
	15		16		18		20		22		24	
	Beef sample 4.	Veal sample 4.	Beef sample 4.	Veal sample 4.	Beef sample 5.	Veal sample 5.	Beef sample 6.	Veal sample 6.	Beef sample 6.	Veal sample 6.	Beef sample 7.	Veal sample 7.
Hours.												
1	5.0	6.5	9.3	7.7	11.4	9.3	9.2	8.4	7.7	6.2	7.8	8.1
2	10.7	11.7	16.9	15.4	20.7	16.6	18.5	16.0	14.5	13.4	13.8	14.0
4	16.5	17.9	[a]28.3	[a]28.1	29.8	25.6	29.1	24.6	23.0	21.1	20.3	19.2
7	22.2	23.5	[a]34.1	[a]34.7	38.7	33.3	37.4	31.5	30.2	26.2	26.1	23.9
24	33.7	34.1	42.9	43.6	47.6	42.2	46.7	40.6	43.7	36.8	36.9	28.5
Theoretical maximum	52.7	46.1	53.2	46.6	53.4	48.3	54.1	44.1	54.1	44.1	50.1	32.5
Extractive nitrogen	7.2	8.4	7.2	8.4	7.2	7.8	8.1	7.7	8.1	7.7	11.9	26.2
Trypsin nitrogen	4.1	4.1	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3

QUANTITY (IN CUBIC CENTIMETERS) OF N/5 ALKALI-PROTEINATE NITROGEN

1	3.7	2.9	3.7	2.0	3.3	2.9	4.0	2.6	3.0	2.3	3.1	3.9
2	6.8	4.2	6.8	5.3	4.2	4.4	4.8	4.0	4.0	6.5	4.7	3.0
4	7.6	5.1	[a]6.2	[a]5.3	4.2	5.7	5.0	5.0	4.3	9.8	5.0	3.3
7	8.6	6.9	[a]5.7	[a]8.2	4.4	7.6	5.5	5.8	4.8	10.6	4.9	2.0
24	6.9	8.3	3.3	4.2	4.3	4.9	3.1	5.1	3.0	6.7	3.6	1.5
Trypsin nitrogen	.....	.....	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Age of meat, days	1	1	9	9	7	7	2	2	21	21	33	33

[a] Results are for five and eight instead of four and seven hours.

In experiments 15 to 24, because of the comparatively large weight of trypsin used, it was desirable to ascertain how much of the trypsin nitrogen appeared in the neutralized digestion filtrate and in the precipitate of alkali proteinate, in order that both may be corrected by the amounts found. Accordingly, two portions of trypsin 1, each weighing 100 mgm., were dissolved in 100 c. c. of 0.5 per cent sodium carbonate and precipitated with 48 c. c. of $N/5$ sulphuric acid, as in the digestion experiments.* The mixtures were heated to a boil and filtered. The total nitrogen ($N/5$) in the filtrates was 4.25 and 4.40 c. c.; in the precipitates, 0.47 and 0.70 c. c. The averages of these are recorded in Table X, and both were used as corrections, as already described on page 691. For trypsins 2 and 3 the term "trypsin nitrogen" in Table XI means the total nitrogen in the trypsin present in the 100 c. c. of digestion fluid. Trypsin 2 contained approximately 90 per cent of its nitrogen as ammonia, and consequently the amount precipitated with the alkali proteinate was disregarded. The results for alkali proteinate in experiments 31 to 34 with trypsin 3 showed that the correction for alkali proteinate derived from the trypsin must have been similar to that in trypsin 1, and the determination of this correction was omitted.

In experiment 18, for example, 100 c. c. of veal sample 5 digestion fluid were neutralized exactly four hours after the digestion began, and the mixture was brought to a boil and filtered. The filtrate contained 37.7 c. c. of $N/5$ nitrogen, of which 4.3 c. c. were derived from the trypsin present and 7.8 c. c. from the extractives present before the digestion was begun; and the figure recorded, 25.6 c. c., is the amount of proteose, peptone, and amino-acid nitrogen actually formed by the digestive process. The precipitated alkali proteinate contained 6.3 c. c. of $N/5$ nitrogen, of which 0.6 c. c. was derived from the trypsin. The corrected figure, 5.7 c. c., is recorded in Table X.

The results with trypsin are practically the same as those with pepsin. They indicate that both the beef and the veal digested with practically the same speed. The presence of only small amounts of alkali proteinate through the experiments indicates that just as soon as the beef or the veal goes into solution as alkali proteinate this is promptly split into the simpler molecules of proteoses, etc.—i. e., the equality in speed of digestion pertains both to the first and to the later stages in the digestive process for both beef and veal. At no time was there any indication that either the beef or the veal contained any nitrogenous substances resistant to the action of the trypsin. In experiments 16 to 24, Table X, approximately 90 per cent of the veal had gone into solution at the end of 24 hours, with similar results for the beef.

In experiments 26 to 34, Table XI, the rate of digestion was measured by both the second and third methods. The comparisons between veal sample 9 and skim-milk sample 2 in experiments 27 and 28 were made for the purpose of ascertaining whether the method used would detect a difference in rate of digestion when such a difference was large. Experiment 28 was a repetition of experiment 27. On account of the comparatively vigorous action of pepsin-hydrochloric-acid solution veal sample 5 in experiment 19 very soon "caught up" with skim-milk sample 1; but in experiments 27 and 28 the striking difference between the rate of digestion of skim-milk sample 2 and veal sample 9 was brought out by the less vigorous cleavage of the trypsin-sodium-carbonate solution. The treatment of skim-milk sample 2 was similar to that of skim-milk sample 1. Skim-milk sample 2 was obtained by skimming, with the aid of a siphon, a sample of ordinary pasteurized milk obtained from a dealer. One gm. of skim-milk sample 2 contained 1.88 c. c. of $N/5$ total nitrogen, or 0.529 per cent. The extractive nitrogen in experiments 27 and 28 was 11.6 and 14.2 per cent, respectively, of the total. The specific gravity was 1.0334 at 26° C. Six hundred gm. of skim-milk sample 2 were weighed into a 2-liter Erlenmeyer flask. The calculated volume of the skim milk was 580.4 c. c. To this 316.4

TABLE XI.—*Rate of formation of proteoses, peptones, and amino acids in trypsin-sodium-carbonate solution*—Continued

QUANTITY (IN CUBIC CENTIMETERS) OF *N*/5 ALKALI PROTEINATE NITROGEN

Digestion period.	Experiment No. —																	
	26		25		27		28		30		31		32		34			
	Beef sample 8.	Veal sample 8.	Beef sample 8.	Veal sample 8.	Skim-milk sample 2.	Veal sample 9.	Skim-milk sample 2.	Veal sample 9.	Beef sample 10.	Veal sample 10.	Beef sample 10.	Veal sample 10.	Beef sample 11.	Veal sample 11.	Beef sample 12.	Veal sample 12.		
Hours.																		
2	5.9	3.7	6.2	5.1	2.9	3.0	3.6	3.0	3.2	3.1	2.8	3.5	3.7	4.9	4.8	5.1		
6	5.0	7.0	5.2	6.5	.9	3.6	.0	5.1	3.9	3.9	4.5	5.0	3.7	3.8	2.3	3.5		
Age of meat ...days.	8	8	31	31	.....	6	.....	21	19	19	28	28	19	19	8	8		
Trypsin used, gm.	1	1	2	2	2	2	2	2	4	4	2	2	4	4	4	4		
Trypsin No.	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3		

It is obvious that the amino nitrogen contained in the digestion fluid and actually determined was the sum of the amino nitrogen derived from (1) the trypsin; (2) the nitrogenous extractives, both of which were present before digestion began; and (3) the amino groups unlinked by the cleavage of the more complex proteoses into the simpler peptones and polypeptids. This is brought about by the action of the trypsin-sodium-carbonate solution during the digestion process. The results actually obtained in the determinations were diminished by the sum of 1 and 2, so that the figures in Table XII correspond to 3, or the amino nitrogen actually formed by the digestion. The minus quantities obtained in this way in some of the experiments for the 15-minute digestion period are probably due to the fact that the errors in determining the small amounts of amino nitrogen in 1 and 2 are large when compared with the small amount formed during 15 minutes' digestion.

DISCUSSION OF THE DIGESTION EXPERIMENTS

THEORETICAL MAXIMUM.—If the digestion of the meat by trypsin could be brought to completion, the meat proteins would be split into simple amino acids. Such a complete cleavage of protein by a trypsin sodium-carbonate solution seldom, if ever, occurs. One reason is that the action of the trypsin becomes slower and slower the nearer the digestion process approaches completion. But by boiling the meat with hydrochloric acid, as already described (p. 678), the proteins and other nitrogenous substances are completely hydrolyzed, or 100 per cent digested. The data in Table XII, under the heading "Theoretical maximum," were obtained from Table V. The total amino nitrogen obtained from hydrochloric-acid hydrolysis minus the amino nitrogen in the extractives gave the figures recorded in Table XII. A slight error was here involved; the correction should have been the amino nitrogen in the extractives after acid hydrolysis, not before. For the present purposes this error is regarded as entirely negligible.

TABLE XII.—*Rate of liberation of free-amino groups in trypsin-sodium-carbonate digestion mixtures*

Digestion period.	Quantity (in milligrams) of amino nitrogen in 10 c.c. of digestion fluid, equivalent to approximately 0.5 gm. of meat in experiment No.—															
	26		25		27		28		30		31		32		34	
	Beef sample 8.	Veal sample 8.	Beef sample 8.	Veal sample 8.	Skim-milk sample 2.	Veal sample 9.	Skim-milk sample 2.	Veal sample 9.	Beef sample 10.	Veal sample 10.	Beef sample 10.	Veal sample 10.	Beef sample 11.	Veal sample 11.	Beef sample 12.	Veal sample 12.
¼ hour			0.08	−0.41	0.56	0.01	0.51	−0.39	−0.22	0.00	0.16	0.16	0.37	0.35	0.44	0.30
1 hour	0.09	(b)														
2 hours	.20	0.25														
3 hours	a .64	a .42	.72	.67	1.93	.30	1.92	.73	1.41	1.42	1.39	1.65	2.76	2.94	3.08	2.89
6 hours	a .79	a .68	1.43	1.26	2.60	1.33	2.33	1.46	2.14	2.32	2.05	2.32	4.16	4.64	4.59	4.45
11 hours			1.94	1.82	2.67	1.73	2.71	1.93								
12 hours									2.09	3.44	2.82	3.51	5.47	6.20	6.12	5.82
24 hours	1.30	1.19	a 2.74	a 2.79	3.36	2.52	a 2.93	a 3.94	3.71	3.92	3.65	5.23	7.87	8.37	8.29	7.93
48 hours	2.13	1.74	2.95	3.68	a 3.81	3.95	a 3.94	a 5.39	4.95	5.39	5.52	6.44	9.37	11.30	9.44	8.93
72 hours	a 2.35	a 2.01	2.09	3.38	4.63	4.92	a 3.82	a 5.14								
96 hours			2.51	4.33					6.11	6.22	6.75	7.40	10.59	13.07	13.36	11.14
120 hours	2.63	2.30	3.04	5.09												
144 hours									a 6.83	7.22	7.46	7.74				
12 days									8.66	8.12			11.97	13.29	12.10	11.45
Theoretical maximum	11.98	10.22	11.98	10.22	(c)		(c)		12.05	10.42	11.97	10.18	12.39	10.94	12.16	.0.89
Amino nitrogen in extractives	.68	.46	.68	.76	1.11	.27	1.11	.27	.51	.53	.59	.77	.43	.41	.50	.47
Amino nitrogen in trypsin	.85	.85	1.71	1.71	1.71	1.71	1.71	1.71	3.42	3.42	.64	.64	1.28	1.28	1.28	1.28
Age of meat.....days	8	8	31	31	.....	6	.....	21	19	19	2.8	2.8	19	19	8	8
Trypsin used.....gm.	1	1	2	2	2	2	2	2	4	4	2	2	4	4	4	4
Trypsin No	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3

a Results obtained one hour later than the time indicated, i. e., in experiment 26, the results were obtained for four and seven hours' digestion, etc.
b Determination lost.
c Low results obtained were rejected (see p. 680).

PERCENTAGE OF MEAT DIGESTED.—In 0.5 gm. of meat the theoretical maximum amino nitrogen varies between 10 and 12 mgm. In order to convert the figures for amino nitrogen in Table XII to the percentage of the total amino nitrogen, it is only necessary to multiply them by a factor easily obtained mentally, which factor varies from 10 to 8.5. Thus, in experiment 32, 10 c. c. of the beef sample 11 digestion fluid contained 4.16 mgm. of amino nitrogen at the end of six hours. At complete digestion 12.39 mgm. would have been present; therefore 4.16÷12.39 or 34 per cent of the total amino nitrogen present had been unlinked by the cleavage of polypeptids under the conditions of the experiment. The same figure may be obtained directly by multiplying in round numbers 4 by 8. A minute before, or after, this particular amino-nitrogen determination was begun, a 100 c. c. portion of the same digestion fluid had been neutralized by the addition of 24.5 c. c. of $N/5$ sulphuric acid. This mixture was brought to a boil in the next few minutes, filtered, and total nitrogen was determined in the filtrate and the precipitate. The results were recorded in Table XI. This table shows that in the same experiment, No. 32, at the end of six hours' digestion of beef sample 11, approximately 60 per cent (i. e., 31.7÷51.2) of the originally insoluble beef sample 11 nitrogenous substances had gone into solution as proteoses, peptones, and amino acids. These figures show how imperfect is the expression "Percentage of meat digested." The digestion process involves several chemical changes which take place at different rates. In general, the cleavage (by trypsin) of the larger molecules of alkali proteinate and proteose goes on at a comparatively rapid rate, the cleavage of the simpler peptone and polypeptid molecules at a slow rate. These facts are illustrated by the foregoing data of experiment 32. By the second method of measuring digestion it was shown (Table XI) that at the end of six hours' digestion 60 per cent of beef sample 11 had been transformed into proteoses, peptones, and amino acids; but by the third method of measuring digestion only one-third of the total amino nitrogen present had been unlinked (Table XII). The last two statements are correct; but it would not be entirely correct to say that according to the second method 60 per cent of beef sample 11 had digested at the end of six hours, or that 34 per cent of beef sample 11 under the same conditions had digested, using the third method of measuring digestion. A single figure can not describe several simultaneous processes in this case. The results in Tables XI and XII were obtained with the same digestion mixtures. The results in Table XII are expressed in milligrams of amino nitrogen obtained from 10 c. c. of digestion fluid, equivalent to approximately 0.5 gm. of meat.

PRESERVATIVES NOT USED.—In all the digestion experiments the flasks in which the meat was heated and later digested were partly sterilized by the heating in the boiling-water bath. During the diges-

tions in which the pepsin-hydrochloric-acid solution was used bacterial action was excluded from the digestion mixtures by the bactericidal action of the 0.2 per cent hydrochloric acid. During the digestions in which trypsin-sodium-carbonate solution was used bacterial action was not excluded, because any bacteria introduced into the digestion mixtures would not be destroyed by 0.5 per cent sodium carbonate. When the digestion period was short (Tables X and XI)—i. e., 24 hours or less—the possible error due to such recently introduced bacteria was negligible because the proteolytic action of the most vigorous proteolytic bacteria is very weak when compared with that of trypsin. When the digestion period was long enough (Table XII) the chemical changes brought about by the bacteria may have appreciably affected the results. No preservatives were used in any of the digestion experiments. This was regarded as an almost necessary condition in view of the fact that both the wholesomeness of immature veal and the influence of certain preservatives on digestion, health, etc., have been subjects of controversy. In the third method it was decided to carry on the digestions as aseptically as possible and to regard the results obtained in the first 48 hours as practically uninfluenced by bacteria. Generally after a few days putrefactive odors were noticed in the digestion mixtures. In so far as a very strong putrefactive odor can be caused by slight chemical changes in which small amounts of strongly odoriferous substances are produced, the amino determinations were made as late as 12 days after beginning the digestion in mixtures that were undoubtedly putrefying as judged by the odor. The practical necessity of a long digestion period in the third method, because of the slowness of amino-nitrogen liberation, together with the indeterminate effect of bacteria, is an objection to this method. The results of the first and second methods showed that under similar conditions mature beef and immature veal proteins were digested to the proteose and peptone stage with practically equal speed. However valuable such data may be they are not complete until the speed of the last transformation in the digestive process is measured for both. If the rate of liberation of amino groups in immature veal had been found to be slower than in mature beef, that fact would have constituted a good reason for the claim that immature veal digests with difficulty in the human digestive tract. The principal advantage of the third method as applied to digestion mixtures lies in the fact that it affords an easy, rapid method of measuring amino-nitrogen liberation, which can not easily be measured by other methods.

GRAPHIC REPRESENTATION OF RESULTS.—In figure 4 the results for amino nitrogen in experiment 32 are plotted. Most of the other curves obtained in this way were flatter because the rate of amino nitrogen liberation by trypsin 2 was slower. The curve for experiment 32 indicates that during the first 36 hours, approximately, the veal digested

a little more rapidly than the beef. After 48 hours the digestion mixture of veal sample 11 smelled putrid. In addition to the amino nitrogen liberated by the trypsin in this mixture non-amino nitrogen was transformed into amino nitrogen by the bacteria. This was indicated by the fact that after 48 hours' digestion amino nitrogen in veal sample 11 was higher than the amount originally present in the meat. During the bacterial and tryptic action which followed, practically all of the nitrogen was transformed to amino nitrogen. The mixture of beef sample 11 did not smell putrid in this experiment. In experiment 34, which was a repetition of experiment 32 except that beef and veal samples 12 were used, both mixtures from these samples had become putrid, and in both, as the data in Table XI show, the amino nitrogen

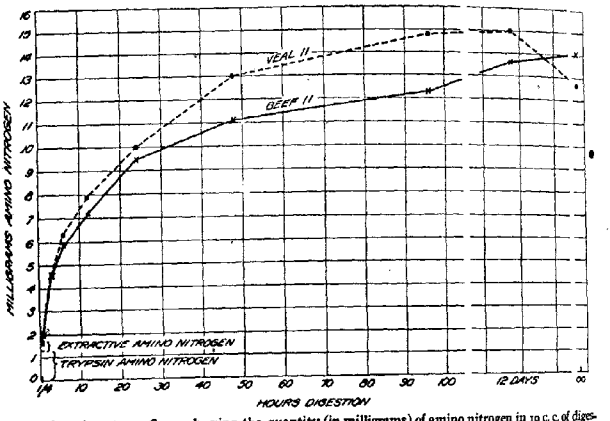

FIG. 4.—Experiment 32. Curve showing the quantity (in milligrams) of amino nitrogen in 10 c. c. of digestion fluid; used 100 gm. of meat plus 2,000 c. c. of 0.5 per cent sodium carbonate plus 4,000 gm. of trypsin 3.

measured was greater in amount than that originally present in the meat. In some of the experiments putrefactive odors were not noticed, although looked for.

The general conclusion drawn from the data of Table XII was the same as that drawn from Tables X and XI—namely, that mature beef and immature veal under the conditions of the experiments were digested by trypsin with equal speed. The slight differences noticed were regarded as physiologically insignificant.

In experiment 27 and its repetition, experiment 28, veal sample 9 was compared with skim-milk sample 2, with the same object as before, to ascertain whether the method would detect a difference in amino-nitrogen liberation where such a difference existed. In both experiments, up to and including the 11-hour determinations, amino nitrogen was liberated in the skim-milk digestion mixtures much more rapidly than in veal sample 9. After this the results were somewhat irregular.

FREE AMMONIA FORMED DURING DIGESTION.—Because of the slowness with which ammonia reacts with nitrous acid (see p. 681) it was desirable to determine the amount of ammonia formed during the digestion of mature beef and immature veal and incidentally to ascertain whether the amounts formed were significantly different for the two meats. In experiments 15, 18, 20, 22, and 24, after 24 hours' digestion, 100 c. c. portions of digestion fluid, containing 0.5 gm. of sodium carbonate and corresponding to 5 gm. of meat, were transferred to Kjeldahl flasks, diluted to 500 c. c. with distilled water, and the ammonia distilled into standard acid. The mixtures were quickly brought to a boil and boiled for half an hour. This method is known to give high results, but for the purpose of comparison the errors were negligible. In all cases except veal sample 7 the ammonia obtained neutralized 2 to 3 c. c. of $N/5$ acid, amounts too small to be a disturbing factor in using the third method or indicating any differences between the beef and veal. From veal sample 7, 7 c. c. of $N/5$ ammonia was obtained. This animal was sick when purchased (see p. 675). On this score the comparatively high ammonia content of trypsin 2 was a disadvantage.

BLANKS ON REAGENTS.—It was found convenient to begin each digestion experiment with fresh alkaline permanganate solution in the absorption pipette and to make blank determinations on the nitrous-acid reagents, water, octyl alcohol, etc., before, during, and after a digestion experiment involving about 20 amino-nitrogen determinations. The blank on the reagents, allowing 20 minutes' reaction time, was 0.6 c. c. nitrogen gas when the permanganate was fresh and rose to 1.2 c. c. after this reagent had been used until absorption had become slow (see p. 680). The smallest volume of nitrogen gas measured in the beginning of a digestion experiment was 3.3 c. c.; the largest, at the end of an experiment, 28.7 c. c.

FEEDING EXPERIMENTS ON CATS

In these experiments cats of various ages were fed on a diet in which immature veal was the sole source of nitrogen.

Osborne and Mendel and their coworkers (1914, p. 334) in their investigations emphasize the difference between maintenance and growth. According to these investigators an animal can not maintain its weight unless the diet contains tryptophan, although the diet may be physiologically sufficient in all other respects. Further, an animal can not grow unless lysin is present in the diet, the amount of growth being conditioned by the amount of lysin available. Conversely, the absence of these unique amino acids results in a decline in weight or in stunted growth. According to McCollum and his coworkers (Hart, McCollum, et al., 1911), a diet properly balanced for growth may not be properly balanced for reproduction—i. e., cows fed on either the whole corn plant or the whole wheat plant would grow, but vigorous calves would be produced only by the corn-fed cows.

The principal object of the feeding experiments was to ascertain whether growth and reproduction were possible on a diet in which immature veal was the sole source of nitrogen. The data of the above investigators were used as a guide in planning the experiments.

DIET.—The cats' diet consisted of immature veal boiled for one to two hours, to which was added filtered butter fat, sodium chlorid, and calcium carbonate. The immature veal was obtained, as already described, from calves seven days old or less which were killed on the premises. When the meat was trimmed for feeding purposes, the lungs, heart, liver, kidneys, and spleen, together with adherent bits of fat, gristle, etc., were included. For the purposes of the analytic work, digestion experiments, etc., the muscle tissue alone was wanted; for the feeding the intention was to include all parts of the veal that ordinarily are eaten. Thirty-four calves were fed to the cats.

At suitable intervals of from four to seven days about 5 kgm. of veal were removed from the containers in cold storage. After being weighed the meat was cut into pieces about as large as ordinary sugar cubes, transferred to an agate-ware kettle containing about 1 liter of hot water, and boiled for one to two hours. The object was to boil the meat in a small amount of water so that it would be convenient for feeding.

Because of the low fat content of the veal, filtered butter fat was added after the boiled veal had cooled. This was obtained by melting several pounds of butter, allowing the water, casein, etc., to settle to the bottom of the containers, and pouring the supernatant fat through filter papers. The butter fat was kept in bottles in cold storage and used as required. According to Osborne and Mendel (1913, p. 424) butter fat contains no nitrogen. Funk and Macallum (1914) found traces of nitrogen in butter fat, which for the purposes of the present consideration of the diet may be disregarded.

No analyses were made of the materials fed. In a few instances the carefully trimmed muscle tissue used for analyses, etc., was included in the veal diet.

Following were the proportions of the various constituents of the diet:

Immature veal	1,300 gm.
Filtered butter fat	45 gm.
Calcium carbonate	10 gm.
Sodium chlorid	10 gm.

The last two constituents were the ordinary "chemically pure analyzed" commercial products. The diet contained no roughage. The above proportions were calculated from the data of Osborne and Mendel (1911, p. 32, 80, 86). Potassium salts and phosphates were omitted, because these were thought to be present in the veal in sufficient amounts.

After the veal had been boiled and the other materials added, the food was kept in an ice box close to the animals' cages. The gelatin present in the food caused the entire mass to become solid, so that there was no loss

through spilling when portions were transferred from the container to the smaller feed pans in the cages. Generally enough food was prepared to last from five to seven days. The ice-box compartment in which the food was kept was also used for the purpose of storing dead guinea pigs, rats, etc., for various biological purposes. Although it was desired to feed the animals with clean food, no unusual precautions were taken. The cover of the can containing the food was seldom tightly in place, and undoubtedly the food was exposed to some extent to bacterial contamination. The conditions under which the meat was kept in cold storage and then boiled were probably better than the conditions in many so-called sanitary kitchens. But the conditions under which the boiled food was stored in the ice box were certainly such as exist in no well-kept kitchen ice box. This was purposely done, in order that the diet actually fed should conform, as nearly as possible, to the poorest rather than the best ice-box conditions for food.

ANIMALS AND ENVIRONMENT.—The animals used in the experiments were ordinary cats, selected at random and brought to the animal room. Some were very young at the beginning of the feeding; others quite old. Their weights are given in Table XIII. After having lived on the immature veal diet for about six months cat 2 was crossed by cat 1, and in due time cat 2 gave birth to a litter of four kittens, given in Table XIII and in figure 6 as cats 5, 6, 7, and 8. One of the kittens (cat 7) died in a few days; the others were nursed by their mother until they could eat the immature veal. It is obvious that since both parents of these kittens had lived and grown on the immature-veal diet for 8 and 10 months, respectively, the birth of these kittens and their subsequent vigorous growth indicated that the diet was entirely satisfactory. There were no indications that toxic bodies were present in the diet or that any of the amino acids essential to normal growth were absent.

TABLE XIII.—*Description of cats used in feeding experiments*

No.	Description.	Weights.			Period of feeding.	Final disposition of animal.
		Initial.	Maximal.	Final.		
		Gm.	*Gm.*	*Gm.*	*Days.*	
1	White male kitten	695	4,080	3,220	473	Chloroformed; autopsy performed.
2	Black female kitten	837	4,040	2,620	408	Do.
3	Yellow male, old	3,605	4,940	4,070	216	Set free.
4	Black male, old	3,350	.....	3,960	50	Returned to owner.
5	White male [a]	[b] 105	.....	3,080	175	Living in a home.
6	White female [a]	[b] 110	.....	2,370	175	Do.
7	Black female [a]	[b] 95	.....	100	15	Died; marasmus.
8	Black male [a]	[b] 105	.....	2,790	175	Set free.
9	Yellow female kitten	580	.....	2,280	114	Do.

[a] Litter produced by cats 1 and 2. [b] At birth.

The animals were kept in cages, singly at first; later, after the kittens had become quite large, they were kept in pairs. The long confinement did not seem to disagree with them. All of the animals were unusually fine in their appearance and disposition, except that toward the close of the experiment cats 1 and 2 apparently suffered from the effects of the long confinement—in their case considerably over a year.

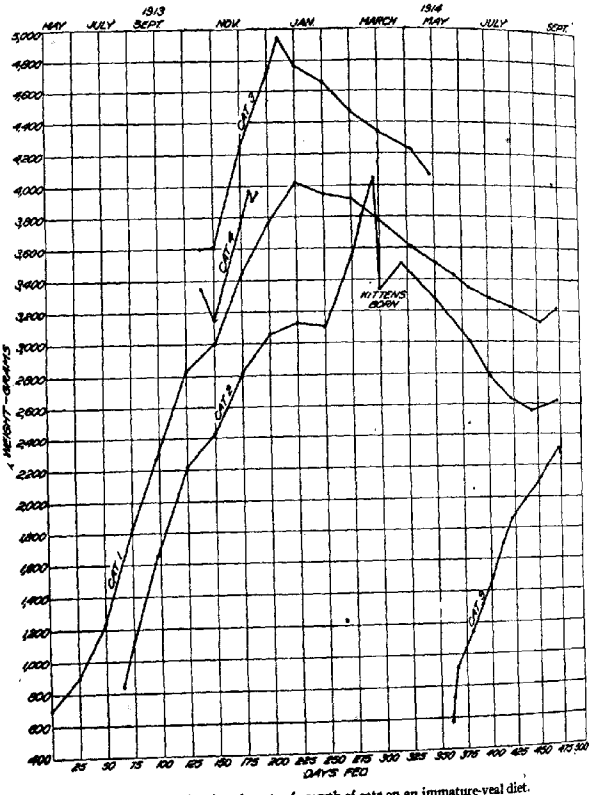

FIG. 5.—Curve showing the rate of growth of cats on an immature-veal diet.

FEEDING.—Twice every day, at 9 a. m. and 3 p. m., liberal portions of the veal food were transferred to the feeding pans and placed in the cages. The animals apparently found the food very acceptable in spite of the monotony of the diet. No attempt was made to regulate the amount of food consumed by any animal; they ate as much as they pleased. All of the boiled veal was eaten; not a single lot of the food was found to be distasteful to the animals or in any way noticeably injurious.

WEIGHTS OF THE ANIMALS.—The animals were weighed twice every week. The rapid growth of the younger animals and the fattening of the older ones are indicated in figures 5 and 6. The reason for the decline in weight of cats 1, 2, 3, and 4 in the spring and summer of 1914 can not be stated with certainty. The fact that cats 5, 6, 8, and 9, all young, gained weight rapidly on the same diet that the other cats were receiving when they were declining in weight indicated that the loss in weight was not due to the diet but rather to a seasonal variation which affected the weights of the older animals. Cats 1 and 2 were chloroformed at the end of the experiment (September 10, 1914) and autopsies performed by Dr.

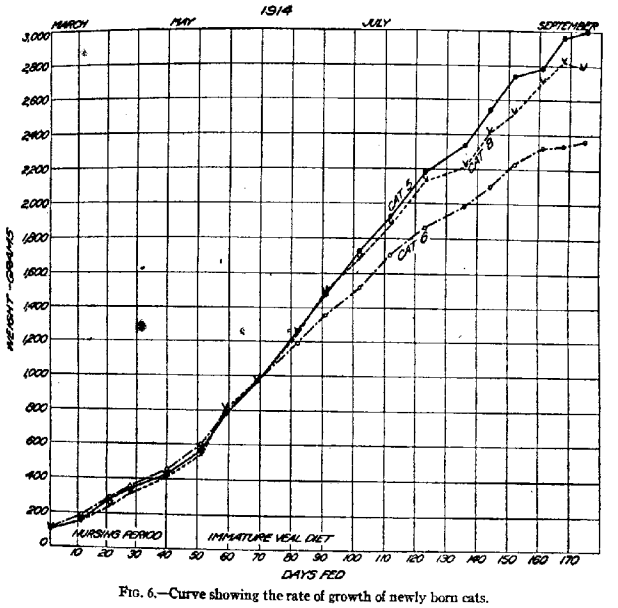

FIG. 6.—Curve showing the rate of growth of newly born cats.

H. J. Washburn, of this division. The animals were found to be in excellent condition, with liberal deposits of fat in both. Apparently the loss in weight in these two animals was due to loss of stored fat. The same was probably true of cat 3, which had the appearance of being unusually fat at the time of its maximum weight.

CRITERIA OF DIETARY SUFFICIENCY.—The excreta of the animals were not collected, nor was any chemical work done directly in connection with the feeding experiments. The ability of the animals to utilize the immature veal for the building of their tissues and for the reproduction and nursing of healthy young animals was regarded as a certain indication that the immature veal contained all the amino acids essential to

maintenance, growth, and reproduction. It is true that only one litter of kittens was born, but this would have been practically impossible had an attempt been made to maintain the parents of these kittens for two-thirds of a year on a diet lacking something essential. Cat 2 went through the period of gestation and nursing with every outward indication of excellent health.[1]

SUMMARY

(1) During the study of the chemical composition of mature beef and of immature veal, no differences between them that are physiologically significant were detected.

(2) In a large number of artificial-digestion experiments immature veal digested as fast as mature beef. The speed of digestion was measured by three different methods.

(3) Cats were fed on a diet in which immature veal was the sole source of nitrogen. The young animals grew normally on the diet; the older ones became fat. A pair of cats, after living two-thirds of a year on the diet, produced a litter of healthy young kittens which, after the nursing period, continued on the immature-veal diet with excellent growth.

(4) The work indicates that immature veal, when properly prepared, is fit for human food, especially when its deficiencies in fat and possibly in small amounts of undetermined constituents are counterbalanced in the ordinary mixed diet.

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[1] The argument has been offered that the metabolism of the fetus and of the newly born is different from that of older animals and that there is a possibility of toxic substances being present in embryonal or young tissues, which substances, though present in amounts too small to be detected by analytic methods, may be very powerful in their action upon the consumer of very young meat; or, as is sometimes alleged, the newly born animal does not excrete its metabolic end products fast enough, with the result that its tissues are loaded with waste material.

The polypeptid nitrogen which passes unused through the assimilatory system of the fetus or of the newly born is, however, not significant. If by any chance the tissues of a very young calf happened to retain some of its own metabolic products because of retarded excretion or from any other cause whatsoever, so long as the animal was normal otherwise there would be practically no danger to the consumer of such meat from poisonous end products of protein breakdown. However, the tissues of very young calves are not loaded with unexcreted nitrogen. The data obtained on this point are direct and conclusive.

(See p. 673.)

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